THE

JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

AND

EUGENE L. OPIE, M.D.

PUBLISHED FOR

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK

VOLUME ELEVENTH

WITH TWENTY-SIX PLATES AND SEVENTY-ONE FIGURES IN THE TEXT

THE NEW ERA PRINTING COMPANY
1909

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THE PATHOGENESIS OF WAXY DEGENERATION OF STRIATED MUSCLES (ZENKER'S DEGENERATION).[1]

By H. GIDEON WELLS.

(From the Pathological Laboratory of the University of Chicago.)

Although there is a considerable literature on various features of the waxy degeneration of striated muscles which Zenker described in connection with typhoid fever, yet there has been nothing definite ascertained concerning the cause of this very striking change in the structure of voluntary fibers. Zenker believed that the cause of the hyaline appearance is an infiltration of an albuminous material from outside the cell, so that all the structures of the fiber are packed and swollen with proteins, thus obliterating the usual structural elements. Later writers have taken the view that the essential process is a coagulation of the myosinogen, although, as Beneke[2] pointed out, in rigor mortis of uninjured muscles there is a coagulation of the myosinogen without the production of anything resembling the characteristic hyaline appearance of waxy degeneration. This author found that involuntary muscle fibers placed in physiological salt solution show areas of hyaline transformation resembling in appearance waxy degeneration, which he attributes to the dissolving out of the muscle proteins that are soluble in salt solution. The hyaline material produced in this way swells up and dissolves quickly when placed in one per cent. of hydrochloric acid or dilute acetic acid, and the change occurs more rapidly if the muscle is immersed in a large volume of salt solution than when it is in a small amount of solution. Erb[3] found that the presence of proteins in the salt solution decreased the rate of development of this form of experimental degeneration, and it

[1] Presented to the Association of Pathologists and Bacteriologists, Ann Arbor, April 17, 1908. Received for publication September 9, 1908.

[2] *Virchow's Archiv*, 1885, xcix, 71.

[3] *Virchow's Archiv*, 1868, xliii, 108.

goes very slowly in blood serum. Whether this sort of hyaline degeneration which occurs in non-striated muscles placed in salt solution is the same as that occurring in pathological striated muscles does not seem to have been determined, but it is difficult to correlate the conditions occurring in muscles in typhoid, acute myositis, etc., with those to which muscles soaked in salt solution are exposed.

Nesti[4] claims that the waxy degeneration of pathological processes occurs after injury of the contractile substance of the muscle fibers when these fibers lie between living fibers, so that a partial absorption of the degenerating proteins is possible; this absorption of the contents of the sarcolemma he considers to account for the typical physical appearance of this condition. Waldeyer[5] noted that the waxy muscle fibers reacted quite the same to various chemical reagents as did normal muscle fibers.

Cohnheim observed that the muscle fibers of the tongues of living frogs may undergo a form of waxy degeneration while being examined under the microscope, and this process has been studied further by Weihl[6] and by Thoma.[7] If a muscle of the tongue of a frog is injured with the point of a needle while being viewed under the microcope, within from three to five seconds two small waxy swellings will usually appear and in a few seconds or minutes spread across the fiber and draw away from one another; similar changes occur when the muscle is cut across. Thoma believes that the waxy appearance is due to the extreme contraction of the injured portions of the fibers, which cause the structural elements of the muscle fiber to be so closely packed together that the striated appearance is lost; the silver-grey appearance of the waxy muscle fibers is probably due to phenomena of refraction and interference caused by the compact striations. Although Thoma considers that this experimentally produced condition is in some respects similar to that occurring in the waxy degeneration of muscles of typhoid and other diseases, yet he points out that the degeneration of typhoid muscles extends for a considerable distance,

[4] *Lo Sperimentale,* 1894, xlviii, 316; abstract in *Cent. f. Path.,* 1895, vi, 215.
[5] *Virchow's Archiv,* 1865, xxxiv, 473.
[6] *Virchow's Archiv,* 1874, lxi, 253.
[7] *Virchow's Archiv,* 1906, clxxxvi, 64.

while the degeneration in the frog's tongue is of very limited extent, and hence the processes may be different.

I have not been able to find any statements concerning the nature of the changes in the muscles in waxy degeneration that are more definite than those quoted above, which suggest several possible explanations of the hyaline appearance, namely: (1) Infiltration with protein until the muscle is made of a uniform homogeneous structure; (2) absorption of part of the muscle proteins, leaving others in a hyaline form; (3) coagulation of the muscle proteins in a homogeneous form: (4) optical phenomena, depending upon a close packing of the striæ-forming elements until the structure appears to be uniform. There is, however, no definite proof that any or all of these processes play a part in producing the typical waxy degeneration of Zenker.

One rather striking fact about this waxy degeneration as we observe it in pathological processes is that it is produced under much the same conditions that give rise to cloudy swelling or coagulation necrosis in other types of cells, *e. g.,* bacterial toxins, ischæmia and trauma. Why the muscle cytoplasm should become *hyaline* from the same causes that make the cytoplasm of other cells *granular* became to me a question of interest, and in discussing the matter with Prof. A. P. Mathews he suggested as another hyaline transformation of proteins the homogeneous swelling which occurs in fibrin that is placed in dilute acids. In view of the fact that muscles are known to produce considerable quantities of sarcolactic acid under certain conditions, and that muscle contains a coagulable protein quite similar to fibrinogen, the analogy seemed very suggestive and I have performed a number of experiments to ascertain whether the lactic acid of the muscle cell might not play a part in producing the condition of waxy degeneration which is so peculiar to muscle cells.

The results of these experiments seem to indicate that this hypothesis is well founded: namely, that the homogeneous transformation of muscle fibers typical of Zenker's waxy degeneration is analogous to the swelling of fibrin in acids, and may be due to the action of the sarcolactic acid formed by the muscle fibers themselves. It hardly seems necessary to give in detail all the protocols of the

experiments, both positive and negative, largely on account of their simplicity. The results may be given briefly as follows:

If small pieces of voluntary muscle, freshly obtained, are placed in physiological salt solution and kept either at room temperature or at 37° C., with toluol to render the materials aseptic, the muscle cells undergo autolysis, and histological examination of fixed and stained specimens removed at different intervals shows a gradual decrease of the staining power of the cytoplasm, the longitudinal striations becoming coarser and more distinct, and eventually the cytoplasm falls into a granular debris. The nuclei persist for a few days, becoming pycnotic and gradually disappearing, while more or less of the striated appearance can be discerned even after the autolysis has continued for a month at room temperature. If heated serum is used instead of salt solution the changes are very much the same, except that the solution of the muscle components is somewhat slower, while when fresh serum is used autolysis takes place much less rapidly, although the structural changes in the muscle are quite the same in character. Evidently, therefore, simple antiseptic autolysis of muscles *in vitro does not* cause changes at all comparable to those of waxy degeneration, but produces rather the ordinary granular disintegration common to all autolyzing cells. In aseptic autolysis accomplished by sterilizing the external surface of large pieces of muscle by scalding, and keeping in sterile dishes while the central parts of the muscle digest themselves, similar negative results have been obtained.

If, on the other hand, the muscle is placed in varying strengths of lactic acid in physiological salt solution, the results are quite different. Even as dilute a solution as $\frac{1}{64}$ *N.* lactic acid causes within an hour or two at 37° C. a distinct swelling of the ends of the fibers, and of the entire length of the most superficial part of the muscle where the lactic acid comes into most intimate contact with the fibers; the swelling is associated with a loss of the transverse and an obscuration of the longitudinal striations. Solutions of $\frac{1}{32}$ *N.* strength cause in from one to three hours a very striking homogeniety of the structure of the swollen fibers, perfectly comparable to the appearances typical of the waxy degeneration seen in pathological muscles. In such homogeneous muscles

the nuclei still stain, although they are smaller and stain more intensely than normal muscle nuclei. If solutions stronger than $\frac{1}{8}$ *N.* lactic acid are used the swelling and loss of striation are less evident, apparently because of a tendency of the strong acid to fix the protoplasm through coagulation. These experiments, repeated with various modifications, and using muscles from dogs, rabbits and man, seem to show that lactic acid possesses the power of causing changes in striated muscle fibers that are microscopically quite similar to those observed in typical Zenker's degeneration. This effect seems to be due to the hydrogen ion, for sodium lactate does not have any such effect, whereas hydrochloric acid causes much the same changes in more dilute solutions, *e. g.*, $\frac{1}{128}$ *N.* hydrochloric acid is about as effective as $\frac{1}{32}$ *N.* to $\frac{1}{16}$ *N.* lactic acid, agreeing with the much greater degree of ionization of hydrochlòric acid in solutions of these strengths.

Similar results may be obtained by injecting solutions of lactic acid in physiological salt solution into the muscles of living animals; there follows the appearance of typical waxy changes at the point where the lactic acid has been forced into the muscle and along the needle tract. The only respect in which this form of waxy degeneration differs from that observed in typhoid is in that the fibers do not tend to break up into short segments so much, but even this effect sometimes appears, and it seems to result oftenest if the injected muscle is not removed for several days, as if the segmentation were the result of postnecrotic changes. In support of the last suggestion is the observation that similar fragmentation of the muscle fibers is observed in muscles undergoing aseptic autolysis for several days, even although no hyaline change has taken place.

It is perfectly possible for the degree of acidity of living muscles to reach an amount equal to that found necessary to produce a homogeneous transformation of the fibers. The most careful study of the development of lactic acid in the muscles has been made by Fletcher and Hopkins,[8] who, however, worked with frog muscle. They found that frog muscles would after heat rigor yield as much as 0.5 per cent. by weight of zinc lactate, or about 0.35 per cent. of lactic acid, which corresponds to about a $\frac{1}{25}$ *N.* solution; nearly

[8] *Jour. of Physiol.*, 1907, xxxv, 247.

as large amounts are obtained if the muscle is merely kept in an atmosphere poor in oxygen. As the metabolism of mammalian muscle is much more active than that of amphibian muscle, it is probable that if anything even greater amounts of lactic acid can be produced in mammalian muscles, especially when the oxygen supply is cut off by thrombi, at the same time that the fibers are stimulated to activity by bacterial toxins.

Although there is no difficulty in producing typical waxy degeneration by injecting bacteria into the muscles of living animals, yet the same variety of bacteria acting upon the dead or excised muscle *in vitro* do not produce waxy changes. Several experiments of this kind have been tried in which staphylococci, colon bacilli and typhoid bacilli, both living and killed by toluol, have been allowed to act upon small pieces of muscle for varying periods in the incubator. Such muscles upon sectioning and staining may be found invaded throughout with bacteria, and yet the fibers show their striations well preserved. Evidently, therefore, it is not alone the action of bacteria upon the muscle that causes the hyaline transformation, but apparently the living muscle itself has something to do with the process.

Simply ligating a muscle, so that it is entirely deprived of its blood supply, does not produce waxy degeneration. There develops a high degree of œdema, particularly in the interstitial tissue, so that although the muscle as a whole is much enlarged the individual fibers are not noticeably swollen and their striations persist until the muscle cells disintegrate. If such ligated and necrotic muscles are chopped fine and extracted thoroughly with hot water, the amount of alkali needed to neutralize the extract to phenol-phtalein is always much less than with similar extracts made from the corresponding muscle of the other limb of the same animal. This negative result is not of much significance, however, for in the first place most of the acidity found in the normal control muscle is probably produced as a result of the stimulation of the muscle during its removal and the process of chopping it up for extraction, as Fletcher and Hopkins have shown, while of course the dead ligated muscle would not produce acid during these manipulations. Secondly, the infiltration of plasma into the ligated muscle would largely suppress

any acidity that might have resulted from the anemia produced directly after the ligation. Indeed, it is the influence of such unavoidable factors as these that have made it impossible to actually determine that there is or is not a development of lactic acid in muscle fibers during the production of the waxy changes. For example, if typhoid bacilli are injected into sound muscles of one limb and there produce typical waxy degeneration, titration of extracts of these muscles shows the same amount of acidity to phenolphthalein as the corresponding muscles of the opposite limb if the titration is performed at once or within three or four hours; but if the muscles are not removed until after twelve, twenty-four or forty-eight hours, when an inflammatory œdema has developed, then the extract from the infected muscle requires less alkali to neutralize it to phenol-phthalein than does the control. It is perfectly possible, and indeed probable, that an infected muscle fiber may at some particular stage of its infection be stimulated violently to production of lactic acid, which causes hyaline changes in the fiber in which it was formed; this acid then diffusing outward would be neutralized by the blood plasma and thus be rendered incapable of detection by chemical means. Therefore, the fact that negative results were obtained in all attempts to demonstrate an increased acidity by titration of muscle in which waxy degeneration had been produced by injection of typhoid bacilli, does not speak against the hypothesis that lactic acid causes the waxy transformation of the muscles in the so-called Zenker's degeneration.

As further evidence that waxy degeneration may be the result of the presence of excessive amounts of lactic acid in the muscles, we have experimental evidence that under conditions in which lactic acid is produced in large amounts by muscles made to contract rapidly and violently until fatigued, a very typical waxy degeneration may appear in the otherwise uninjured muscle fibers. This was demonstrated by the following experiments.[9] The sciatic nerve was isolated in rabbits and submitted to repeated stimulation with induced current, so regulated that the force and frequency of shocks was just short of throwing the muscle into tetanus. This was continued for about an hour, until no more satisfactory contractions

[9] For assistance in these experiments I am indebted to Mr. J. R. Greer.

could be secured on account of exhaustion of the muscle or the nerve; the muscles were then quickly removed and small pieces dropped into Zenker's fluid (without acetic acid). The stimulated muscle appeared paler than the normal muscles, and in places seemed to be almost waxy. Histologically, such muscle, which had been stimulated to the maximum for about one hour, showed very strikingly and typically the appearance of waxy degeneration. Frequently an entire section would show practically no fibers that were not more or less affected, but usually the changes were somewhat focal, and resembled perfectly the changes seen in degenerating muscle. Most of the fibers in such areas appear swollen, perfectly homogeneous, and entirely devoid of either transverse or longitudinal striations; and some show transverse clefts increasing the resemblance to Zenker's degeneration. In such fibers the nuclei appear somewhat smaller and more deeply stained than normal. Not a few fibers show a peripheral zone which stains deeply and in a diffuse manner with hematoxylin; as hematoxylin is a basic stain with a chemical affinity for acid tissue elements, it would seem possible that these deeply staining areas in the fibers represent places where an excessive amount of acid protein or syntonin-like substances have been formed by the action of lactic acid upon the muscle proteins.

In order to decrease the oxidation and if possible to increase by this means the formation of more lactic acid in the muscles, the same experiment was repeated after isolating the muscles from their blood supply by ligatures which did not include the nerves. Such muscles, however, could be made to keep up their contraction for a comparatively short time, about fifteen minutes, and so the final result was if anything less degeneration than was obtained with the muscles with intact blood supply, which had contracted for an hour before exhaustion. While there is no positive evidence that the hyaline transformation observed in these exhausted muscles is due to the lactic acid formed in their active metabolism, rather than to some other unrecognized cause, yet taken with the facts previously brought out and the fact that muscles thus stimulated are known to produce a large amount of lactic acid, the evidence seems to be strongly in favor of associating the lactic acid and the hyaline transformation as cause and effect.

SUMMARY.

In view of theoretical deductions and the positive results obtained in the above experiments, it would seem probable that the production of waxy degeneration depends upon the action of lactic acid which is formed by the living muscle under the stimulation of infecting bacteria or their toxins, the formation of large amounts of lactic acid and its accumulation being perhaps favored by defective circulation through the injured muscle. The hyaline transformation of muscle acted upon by lactic acid is analogous to the swelling of fibrin placed in dilute acids. This view is supported by both negative and positive experimental evidence—the negative evidence being that simple anemic necrosis, aseptic or antiseptic autolysis whether *in vivo* or *in vitro,* or the action of bacteria of various sorts on muscle *in vitro,* are all incapable of causing changes in muscle cells resembling those characteristic of waxy or hyaline degeneration of striated muscle. The positive evidence consists in the demonstration that lactic acid, even in dilutions comparable to the amounts that can be formed in living muscle, can produce a similar or identical waxy transformation of the striated muscle fibers, both *in vitro* and *in vivo;* and also the observation that muscles stimulated to exhaustion, under which condition lactic acid is known to accumulate in the muscle, show microscopically changes identical with those of Zenker's waxy degeneration.

THE POSTMORTEM RIGOR OF THE MAMMALIAN HEART AND THE INFLUENCE OF AN ANTE-MORTEM STIMULATION OF THE PNEUMOGASTRIC NERVES UPON ITS DEVELOPMENT.[1]

By DON R. JOSEPH and S. J. MELTZER.

(*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*)

Part I.

RIGOR MORTIS OF THE HEART.

The main object of the investigations to be reported in this paper was the study of the influence of electrical stimulation of the pneumogastric nerves upon the onset and development of postmortem cardiac rigor in mammals. We shall state later the considerations which led up to the investigation of this problem. However, the study of the changes which cardiac rigor may undergo as a result of stimulation of the vagi, presupposes a definite knowledge of the course of cardiac rigor under so-called normal conditions. Here we were soon confronted with the fact that such knowledge is still far from being complete. In pursuing our main studies we were, therefore, obliged to study carefully at the same time the course of cardiac rigor in animals whose vagi were not stimulated. We have thus collected a number of facts pertaining to the onset and development of rigor of the mammalian heart in normal animals. These observations we intend to embody in the present paper. Before giving the report of these findings we wish to review briefly the experimental data upon which the present knowledge of cardiac rigor is founded.

Historical.—Our knowledge of cardiac rigor is, as stated before, far from being complete. While the studies of rigor of the skeletal

[1] Received for publication September 18, 1908.

muscles are very numerous and date back at least a hundred years, rigor mortis of the heart has been studied but slightly, and its systematic investigations are practically of only recent date. The obvious reason for this difference is this: whereas general rigor presents itself after the death of man and beast without any special preparation, the condition of the heart can be observed only after special dissection. Furthermore, autopsies being usually made only for special purposes—pathologic or forensic—were frequently performed some time after death, when the rigor had perhaps passed off. Then, too, the character of rigor which permits of hardly any other interpretation when it is present in skeletal muscle, is capable of being confounded with other states when it occurs in the heart. Rigor mortis is characterized by a contraction; a shortening of all the dimensions of the heart; and by a thickening of its walls. These features, however, may be met with also either in so-called concentric hypertrophy of the heart, or when the heart remains in systole at death. As long as fifty years ago Kussmaul called attention to the possible confusion of rigor with either of these states, the actual existence of which he doubted. He thought concentric hypertrophies as well as cardiac systole after death were possibly nothing but cardiac rigor. Systematic studies of rigor mortis of the heart began only about two decades ago.

Strassmann[2] investigated in 1889 the question of whether in some cases the heart may indeed stop in systole during death. He examined the amount of blood present in the right and left ventricles in a number of dogs killed by potassium cyanide, asphyxia, exsanguination, etc. Examinations were made in some cases immediately after death, and in still others after twenty-four hours. He came to the conclusion that during death the heart stops in diastole, and that the heart muscle gets into a state of rigor which sets in quite early. In a later study,[3] 1896, he compared the dimensions of the hearts of dogs immediately after death, and twenty-four hours later. The measurements were taken by means of drawings and by the insertion of needles into various parts of both ventricles and comparing the distances between them as they presented themselves at each examination. The conclusion drawn from these observations was that during the development of rigor the heart becomes smaller in all dimensions.

Brouardel,[4] 1889, studied the condition of the hearts of animals post-mortem,

[2] Strassmann, *Vierteljahrssch. f. gerichtl. Medizin*, 1889, Ser. 2, li, 300.
[3] Strassmann, *idem.*, 1896, Ser. 3, xii, Suppl., 110.
[4] Brouardel, *Gazette des Hopitaux*, 1889, lxii, 653.

by connecting them with a manometer. The main object of the investigations of Brouardel as well as of Strassman seems to have been to prove from a forensic point of view, that the heart after death passes into a state of rigor—and this less than twenty years ago.

Meirowsky,[5] 1899, reports the experiments of Ludloff on heart rigor which were carried out in the physiological laboratory of L. Hermann. Hearts of cats and rabbits were removed from the body, all blood vessels ligated, except the aorta, into which a cannula was tied, and after filling the ventricle and tubes with a physiological salt solution, they were connected with a manometer containing chloroform. The hearts were kept at various temperatures in solutions of normal saline. The level of the colored chloroform in the manometer was observed every few minutes. According to Meirowsky the beginning of the reduction of volume of the ventricle, as shown by the manometer, which was taken as being exactly proportionate to the onset of rigor, begins in most cases immediately after death, and attains its maximum at the earliest in fifty minutes, and at the latest in two and a half days.

R. F. Fuchs,[6] 1900, removed the heart from the body, tied cannulas in the ventricles through the aorta and pulmonary artery and in the auricles through the corresponding veins. He found that soon after death, solutions injected through the ventricles would pass unhampered through the auricles, which showed that at this period the atrio-ventricular valves were patent About an hour and a half later no liquid could pass from the ventricles to the auricles. Fuchs assumed that this impassability was due to the development of rigor of the heart. A similar observation was previously made by L. Krehl,[7] 1889. Fuchs seems to have been the first to establish the fact that rigor of the heart appears before the occurrence of general rigor, and he ascribes it to the preceding continuous activity of the heart.

MacWilliam,[8] 1901, was the first to publish a tracing presenting the development of rigor of the left ventricle. The tracing was obtained by tying a cannula into the aortic orifice of the heart of a cat just excised, and connecting the cannula with a mercurial manometer, the oscillations of which were transmitted to a slow-moving drum. The pressure within the ventricle, caused by the contraction of the rigor, may, so MacWilliam states, become as high as 25 to 30 mm. Hg. and may be maintained at this high level for a considerable time. No special data are given for the time of onset, and further development of the rigor after death. Judging by the tracing, it seems that the maximum of rigor is attained in less than an hour and a half, and that at least one-third of the contraction occurs almost immediately after connection with the manometer, since a good part of the ascending limb of the curve is nearly vertical. In this particular case it is not stated how soon after death the ventricle was connected with the kymograph. MacWilliam points out that there is no special parallelism between cardiac and skeletal muscle in regard to the occurrence of rigor, its intensity, etc. Irritability may still persist after the beginning of

[5] Meirowsky, *Pflüger's Archiv,* 1899, lxxviii, 64.
[6] R. F. Fuchs, *Zeitsch. f. Heilk., Abt. f. pathol. Anat.,* 1900, i, 1.
[7] L. Krehl, *Arch. f. Anat. u. Physiol., Physiol. Abt.,* 1889, 253.
[8] MacWilliam, *Jour. of Physiol.,* 1901–1902, xxvii, 336.

cardiac rigor and some slow and rapid oscillations occur in some hearts during the course of rigor.

An extensive investigation on post-mortem changes in the form of the heart was carried out by J. Rothberger,[9] 1903. He examined the hearts of two hundred dogs which were killed in various ways or which had died from various causes. The hearts were removed from the body, the blood content washed out by a physiological salt solution and cannulas tied in the vena cava, left auricle and aorta. The ventricles and connecting tubes were filled with salt solution and connected with a manometer containing water. By means of a float, the changes of level of the water in the manometer were marked on a slow-moving drum. In a number of experiments, hearts taken immediately after death, and twenty-four hours later, were hardened in formalin; four circular sections were made of each, and by drawings, the size and outlines of these sections of the ventricles were compared. Rothberger brought out numerous details of which we shall mention here only the following few statements.

All hearts are, during death, in a state of diastole; some, however, are very relaxed, wide and filled with blood; others are also relaxed, but are small and empty. The latter hearts are slightly tonically contracted. This difference in the behavior of the hearts depends upon the different modes of death. The tracings obtained show various phases. Immediately after filling the heart and connecting it with the kymograph, a rapid rise occurs which is followed sooner or later by a primary fall; then another rise occurs to be followed finally by a secondary fall. The first rise, Rothberger thinks, is a vital phenomenon—a tonic contraction brought on by distension of the heart through the filling of its cavities, and aggravated by the increased irritability of the ventricles, due to the stimulating effect of the solution of sodium chloride. With the gradual loss of irritability the tonus subsides and the primary dilatation takes place. Then the rigor sets in and the secondary rise appears. Finally the resolution of the rigor is the cause of the secondary fall. Rothberger believes that a similar sequence of events takes place in the heart after death, even when it remains under natural connections within the chest cavity. He assumes, with Bayliss and Starling,[10] and with MacWilliam,[11] that at the time of death there remains enough positive intravascular pressure to distend the heart and thus cause, soon after death, a tonic contraction. This contraction, however, under natural conditions, passes over into rigor without an intervening primary dilatation. According to Rothberger, then, the heart very soon after death becomes contracted, but this contraction which develops quite abruptly is a "vital" phenomenon and is brought on by distension. With the loss of irritability of the heart, the "vital" contraction gives way and passes over gradually into the developing rigor. The rigor systole, according to Rothberger, is less strong than the preceding one and appears, Rothberger states, in most cases, two or three hours after death.

This is, in brief, a review of all the systematic investigations made on the subject of cardiac rigor, as far as we know them.

[9] Rothberger, *Pflüger's Archiv*, 1903, xcix, 389.

[10] Bayliss and Starling, *Jour. of Physiol.*, 1894, xvi, 166.

[11] MacWilliam, *loc. cit.*

Neither investigations nor the established facts are very numerous. All writers agree that during death the heart stands still in diastole and all seem to agree that in general the rigor sets in early after death. As to the exact time, Meirowsky (Ludloff) states that the rigor begins in most cases immediately after death, and the tracing of MacWilliam seems to agree with this statement. Fuchs gives the time for the beginning of rigor as the middle of the second hour, and Rothberger speaks of two to three hours. Meirowsky, MacWilliam and Rothberger employed practically the same method —the connection of the cavity of the heart with a manometer. There is one point which comes out with uniformity in all three investigations—namely that soon after the manometer is connected with the cavity of the ventricle, a fairly abrupt rise in the manometer occurs indicating a contraction of the ventricle. Meirowsky and MacWilliam ascribe this to the onset of rigor. Rothberger, who apparently studied the question more extensively, assumes that the contraction occurring immediately after the connection with the manometer is not rigor, but is a vital phenomenon, artificially brought on through the distension of the ventricle by filling it with the solution of sodium chloride. This interpretation of the phenomenon seems to be plausible indeed. But then Rothberger assumes further that such a " vital " contracton takes place also in the normal connections of the heart within the dead body, and this because there is, according to him, at the time of death enough intravascular pressure to cause a contraction similar to that which occurrs when connecting the excised heart with the manometer. This last assumption contains very unusual elements. In the first place, it would distinguish the rigor of the heart strikingly from the rigor of a skeletal muscles, inasmuch as the rigor of the latter is not preceded by any "vital" contractions except under the influence of certain poisons. In the second place, this assumption would practically deny the statement that the heart is in diastole during death. Rothberger has seen the vital contraction setting in immediately after connecting the ventricle with the manometer, which was about fifteen minutes after the death of the animal, because, as Rothberger states, it took so long to make all the necessary preparations. In the normal connections of the heart, such a " vital " tonic con-

traction ought to occur immedately after death, since, according to Rothberger himself, there is enough intravascular pressure at death to produce such a tonic contraction. This would mean that at death immediately after the last normal diastole the heart would contract and remain tonically contracted. But this means, in other words, that at death from any cause the heart stands still in a state of systole, lasting often over two hours. We cannot see how Rothberger can avoid drawing such a conclusion from the second assumption which he makes.

The results, therefore, which were obtained by Meirowsky (Ludloff), MacWilliam and Rothberger by means of a manometer can hardly be accepted as presenting the actual conditions as they occur in the heart normally connected within the body.

The investigations of Brouardel, Ludloff, Fuchs, MacWilliam and Rothberger were carried out by means of connecting the ventricle with a manometer. This method, as we have seen, introduces an artificial element which permits of more than one interpretation. Strassmann, who studied the appearance and outlines of the heart while it remained undisturbed within the thoracic cavity, has seen each heart at the utmost twice for a brief time. He could only state that there was a change of form at a certain time, but was unable to state when rigor sets in and how long it takes until it reaches its maximum.

For the rigor of skeletal muscles which is plainly visible without any special preparation, simple observation was amply sufficient to establish firmly the main facts of the phenomenon. Would not such a simple method be of great advantage also in the study of cardiac rigor? It is possible that the heart was watched by one or the other of these observers in its natural connections within the body for many hours after death, but there is no record of it—at least such a record did not come to our knowledge.

Our Own Observations.—As stated in the introduction, the chief object of the present research was to study the effect of vagus stimulation upon the course of cardiac rigor. Our studies of cardiac rigor under normal conditions were made only incidentally from the necessity for having careful extensive observations upon control animals. In the present research each experiment consisted

in parallel observations made on the onset and development of the rigor of both ventricles simultaneously in two animals: one whose vagi were stimulated and another whose vagi remained untouched. Forty-six double experiments of this kind were made, which means that the course of normal cardiac rigor was studied in forty-six animals. The foregoing analysis of the status of the present knowledge of our subject makes it evident that further studies of it are very desirable. Our report will demonstrate, we believe, that the method of simple observation is as well capable of shedding light upon the normal course of cardiac rigor, as it has been upon the rigor of skeletal muscles.

Method.—The control observations were made on five rabbits, fifteen cats and twenty-six dogs. With three exceptions death was brought about in all animals by the same method—namely exsanguination. The animals were etherized, tracheotomized and both carotid arteries exposed and severed. When bleeding ceased and the convulsions and respiration had stopped completely, the sternum and large sections of most of the ribs were rapidly removed, the pericardium freely dissected away, and the heart thus rapidly exposed to full view. In a number of experiments the animals were etherized for thirty to forty-five minutes, and longer, in order to be comparable with the animals whose vagi were stimulated for half an hour or more, while under the influence of ether. In such cases ether was administered through a tracheotomy tube.

In a few instances we made an attempt to study the cardiac rigor by the graphic method. The results were very unsatisfactory. The results which we are going to state here were obtained by direct observation, *i. e.*, by ocular inspection and by palpation. In thirty-nine cases the heart remained throughout the entire time of observation in its natural position and connections within the animal body: in six experiments (on dogs) the heart, after ligating all the blood vessels, was excised and suspended in a transparent bottle filled with Ringer's solution or a solution of 0.9 per cent. sodium chloride. From the time of exposure the heart remained under constant observation for many hours, or until the rigor reached its maximum. Except in a few cases we have made no special study of that stage when the rigor goes into solution. The criteria

by which we judged the occurrence of rigor were the changes in size and shape, and the changes in consistency tested by palpation. The change in color we found to be an unreliable criterion. According to the rapidity of the course of development of rigor the heart was tested more or less often, but rarely oftener than once every two minutes, or less often than ten minutes. In many experiments the heart was covered in the interval between observations with a very thin layer of absorbent cotton, saturated with a solution of sodium chloride or Ringer's solution. In other experiments the hearts remained uncovered, but were moistened with Ringer's or saline solution now and then to prevent drying. The room temperature during the entire course of this investigation varied between 19° C. and 25° C.

From our extended experience we can make the positive claim that, as in the case of skeletal muscle rigor, the method of direct observation gives most satisfactory results and permits the drawing of conclusions in a safer way than the method of connecting the cavities of the ventricle with a manometer. The changes in the diameters of the heart and in its configuration indicate satisfactorily the onset, progress and maximum of the rigor. A still better indicator is the change in the consistency of the heart muscle. By palpation one is capable of reliably recognizing the beginning of the change as well as its progress. This is especially true after one has acquired some experience with this method.

We may add that our data were derived from observations made by two observers independently of each other.

Results.—For most of our experiments we may divide the interval between the death of the animal and the attainment of maximum cardiac rigor into three periods. Before going further we wish to say that we considered the animal to be dead when the respiration had ceased completely; bleeding and all convulsions usually subsiding before. Furthermore, after respiratory death, some time was taken up by the opening of the thorax and the proper exposure of the heart. This time is included in the first period. The three periods are (1) the period during which the heart was still pulsating, (2) the period during which the heart was quiet and relaxed, and (3) the period taken up by the devel-

opment of rigor. We shall employ these periods as a basis for the report of our observations.

PULSATION OF THE HEART AFTER DEATH.

In every one of the control (normal) animals the heart continued beating after stoppage of the respiration, and in nearly all of them the heart was found beating on opening of the thorax. As a rule all compartments took part in the beating which first presented itself. We shall, however, deal with the right and left ventricles separately, since there is quite a marked difference between the two with reference to the length of time during which the pulsation continued.

Left Ventricle. Dogs.—For the twenty-six normal dogs the average time during which the left ventricle continued to beat after respiratory death was twenty-seven minutes. This average includes also the six hearts which were suspended in Ringer's solution or normal saline. The average time for the twenty hearts which remained *in situ* in the thorax was about ten minutes—two minutes being the shortest and twenty-two minutes the longest time the left ventricle was beating after death.

The average time for three hearts (of dogs) which were suspended in normal saline immediately after death was sixty-nine minutes, and for the three hearts suspended in Ringer's solution about ninety minutes. These hearts were not washed out at all and the blood vessels were ligated before suspension of the hearts in the solution, so that none of the solutions could enter the cavities of the heart or the coronary arteries.

While the number of three experiments for each solution is too small to give sufficient weight to the absolute figures thus obtained, these experiments nevertheless demonstrate clearly enough the considerable influence which a bath in a salt solution is capable of exerting upon the vitality of the excised hearts. And we may point out again that since the cardiac vessels were all ligated, the solutions could reach the tissues of the heart only through the epicardium.

Etherization.—Of the twenty dogs whose hearts remained in the thorax fourteen received ether for at least thirty or forty minutes longer than the remaining animals. Grouping the animals

according to this principle we find a marked difference between averages of the two groups. While the average time for the ether dogs amounts to about fourteen minutes, the hearts of the dogs which received ether for only a short time, continued to beat for an average of only about six minutes. Could the prolonged etherization have had the effect of prolonging the pulsation of the left ventricle? We shall draw no positive conclusions from this meagre observation, nor shall we enter into a discussion of this point. We wish, however, to call to mind here that a good many practitioners have employed subcutaneous injections of ether as a cardiac stimulant.

Cats.—In fifteen normal cats the average time during which the left ventricle continued to beat amounted to about fourteen minutes. In this average are included seven cats which besides the usual etherization received atropine. Their average alone, *i. e.*, that of the atropinized animals, amounts to thirteen minutes, which increases the average of the remaining eight cats to a little over sixteen minutes.

Rabbits.—The average duration of the pulsation of the left ventricle in five rabbits amounts to thirty-two minutes. Four of these rabbits were killed by asphyxia and only one by bleeding. In this one the left ventricle continued beating for forty-seven minutes. The asphyxia consisted in three in opening the chest freely under ether and one animal was killed by pithing which means perhaps asphyxia plus internal bleeding. In this animal the left ventricle continued beating for thirty-six minutes.

Right Ventricle. Dogs.—The average duration of the pulsation of the right ventricle for all the twenty-six dogs amounts to forty-five minutes. Excluding the six animals whose hearts were suspended in solutions, the average for the remaining twenty hearts is about thirty-one minutes. Dividing these animals again according to the duration of etherization the average for the fourteen dogs which received ether for thirty or forty minutes and longer amounts to about thirty-five minutes. The average for the remaining six dogs is only about twenty-one minutes. The average time for the right ventricle of the three hearts suspended in Ringer's solution amounts to about one hundred and thirty-one minutes, and for the hearts suspended in a physiological solution of sodium

chloride it amounts to sixty-four minutes. Here again there is a striking difference between the hearts remaining *in situ* and those suspended in a salt solution; and the difference between the hearts suspended in a Ringer's solution and those suspended in a physiological solution of sodium chloride, is for the right ventricle even greater than for the left. The favoring effect of a prolonged etherization comes out quite distinctly also in the duration of pulsation of the right ventricle.

Cats.—For the right ventricle we have notes on only fourteen cats. The average time for the entire number amounts to forty-three minutes. For the seven atropinized cats the average time of pulsation amounts to only thirty-three minutes. This increases the average time of the remaining seven non-atropinized cats to fifty-three minutes.

Rabbits.—For the length of time during which the right ventricle continued beating in rabbits we have complete notes in three experiments only, and those are for the rabbits which were killed by asphyxia. The average time here amounts to fifty-one minutes.

The various data regarding the average time of pulsation of left and right ventricles after death are embodied in Table A, which follows:

TABLE A.

Shows the Average Time the Left and Right Ventricles Continued Beating after Death from Exsanguination in Dogs, Cats and Rabbits.

(L. V. = left ventricle; R. V. = right ventricle.)

Number and Species of Animals.	Conditions of Experiment.	L. V.	R. V.
26 dogs.	Total of dogs.	27 minutes.	45 minutes.
20 dogs.	Total of dogs, with heart *in situ*.	10 "	31 "
14 dogs.	Hearts *in situ* and prolonged etherization.	14 "	35 "
6 dogs.	Hearts *in situ* and short etherization.	6 "	21 "
3 dogs.	Hearts suspended in Ringer's solution.	90 "	131 "
3 dogs.	Hearts suspended in 0.9 per cent. NaCl.	69 "	64 "
15 cats.	Total of cats.	14 "	(1) 43 "
7 cats.	Atropinized.	13 "	33 "
8 cats.	Non-atropinized.	16 "	(2) 53 "
5 rabbits.	Total of rabbits.	32 "	(3) 51 "

(1) 14 cats only. (2) 7 cats only.

(3) Complete notes only on three rabbits.

The above table brings out the difference between the left and right ventricles. In all conditions except one the right ventricle continues beating considerably longer than the left. The exception occurred when the entire heart was suspended in a 0.9 per cent. solution of sodium chloride.

The table brings out some more facts of interest. It shows in the first place that in dogs which were subjected to a longer etherization the left as well as the right ventricle continues to beat longer than the ventricle of dogs which had undergone only a brief etherization. Furthermore it shows that in atropinized cats both ventricles stop beating sooner than in non-atropinized. Finally there is a striking difference between the hearts (of dogs) which remained *in situ* and the hearts which were suspended in a salt solution. In the latter case both ventricles continued to beat much longer than in the non-excised hearts.

During the period of pulsation after death the ventricular beats do not, of course, remain of uniform rhythm and strength. They gradually diminish in vigor and frequency. The rate may fall as low as half a dozen beats a minute or even less—the contractions becoming gradually very weak and slow.

We made the interesting observation that the pulsations do not stop in the entire ventricle at once. The part nearest the base stops first; the entire ventricle is seen to be pulsating except the basal portion. Then the quiescent part gradually increases in extent downward until only the apex contracts rhythmically. Later only the very tip of the apex continues beating alone, and finally this stops also. Both ventricles show the same course: the uppermost part stopping first and the lowermost part stopping last. The successive dropping out of the various parts of the ventricles from regular participation in the systolic contractions takes place gradually and spreads much slower in the right ventricle than in the left. The entire process is more sharply pronounced in the left ventricle than in the right. We have to add that with regard to the latter we find in one instance a note reading: "the last part to stop beating was the portion nearest the right auricle."

In a number of cases we observed that the last motion to occur was in the form of fibrillary contractions. It is noteworthy that

the fibrillary contractions occurred mostly in the right ventricle, and the two instances in which fibrillary contractions appeared in the left ventricle occurred while the heart was suspended in a solution of sodium chloride, the right ventricle also showing fibrillary contractions.

Auricles.—For various reasons we were obliged to neglect the systematic study of the behavior of the auricles. We have, however, collected some data, part of which may be given here. In most cases the auricles seem to beat longer than the ventricles, and the usual order in which they stop beating seems to be: L. V., R.V., L.A., R.A. The right auricle frequently beats very long. In the heart of a rabbit we observed the auricle to pulsate for more than twenty-four hours after death.

When the activity of the heart began to lose its vigor, the phenomenon of heart block sometimes made its appearance, *i. e.*, both ventricles continued to beat rhythmically, but only with every second, third or fourth auricular beat. We have noted occasionally such unusual rhythms as 1:10 and 1:20. However, such heart blocks in which both ventricles were concerned simultaneously were rare. More frequently the left ventricle would beat rhythmically with every second, third, etc., beat of the right ventricle and the two auricles together. We have notes on instances in which the left auricle and ventricle were pulsating properly together, but both beating synchronously only with every second beat of the right auriculo-ventricular rhythm. We have further notes in which the left auricle stopped beating, while the left ventricle continued beating in the previous rhythm; also a note on a similar relation between the right auricle and ventricle; and finally notes on instances in which both auricles stopped beating long before both ventricles. In short, during the cardiac pulsations after death various dissociations take place between the auricles and ventricles. A systematic study of these dissociations might perhaps be of assistance in the study, during life, of the phenomena of dissociation of the normal cardiac rhythm.

In reporting above that, on opening the chest, the heart was found beating, we expressed it by saying that the heart was found beating in "nearly" all the control animals. There were a few exceptions to the rule. These exceptions are as follows: In Rabbit A (control), of Experiment 4, artificial respiration was used, and on stopping it the respiratory convulsions set in. When these ceased the heart was found to have stopped too. But two minutes later the heart began to beat again and continued to beat for over ten minutes. In Rabbit A (control), of Experiment 5, the heart was beating when it was first seen and it stopped after one minute. However, after thirty seconds, the heart began to beat again.

In Dog A, of Experiment 36, twelve minutes passed after respiratory death before the heart was completely exposed. It was then found to be already quiet.

In Dog A, of Experiment 39, the left ventricle had stopped when it was first completely exposed, which was about five minutes after respiratory death.

A behavior different from the other hearts was observed in those which were immersed in salt solutions. One heart was found quiet when first seen; the beats of another heart were at first very feeble; in a third the beats were

feeble and became rapidly slow; and a fourth heart, after an initial pulsation stopped beating completely. In all four cases the *ventricles soon began to beat again and became gradually fairly vigorous,* the pulsations lasting, as stated above, much longer than in the other control animals whose hearts were left *in situ.* In one instance the pulsations appeared in groups of ten or more with a pause of considerable length between each group. While the hearts which remained *in situ,* without exception, gradually decreased in vigor and frequency, nearly all of the hearts in the salt solution improved for a certain period in both vigor and frequency of pulsation.

PERIOD OF RELAXATION.

Judging by the available data in the recent literature reviewed above, it would appear that at least in the great majority of cases the heart, soon after death, passes into a state of contraction or rigor without the intervention of an appreciable period of relaxation.

From our observations upon normal animals we must claim that just the opposite is true. In the great majority of our control animals there was a definite, fairly long period in which the ventricles were completely relaxed. For the twenty-six dogs there were only two exceptions: for the fifteen cats the exceptions were one for the left ventricle and five for the right ventricle. The records on the five rabbits do not permit of such a summary statement. By the " period of relaxation " we mean the period in which the last trace of a spontaneous movement of any form in any part of a ventricle was gone, and no sign of a beginning of rigor had yet made its appearance in any part of the same ventricle. This does not preclude the condition that one ventricle may be at complete rest while the other may be still beating or be already even in advanced rigor. There were however such periods in a goodly number of cases in which both ventricles were completely relaxed at the same time. The appearance of the heart in absolute relaxation is very characteristic. It looks broadened and flattened. Especially is the transverse diameter visibly larger and the anteroposterior diameter strikingly shorter. *The heart in this intermediate period is distinctly more relaxed than at any diastole of a fairly normal rhythm.* This complete relaxation, however, does not come on abruptly. It develops gradually with the gradual slowing down of the rhythm. What was said above of the entire heart is true

also of each ventricle; they flatten down by their own weight when they are completely relaxed. It is also true for parts of a ventricle. The flabbiness of the upper (basal) part of a ventricle while the lower (apical) part is contracting is an instructive phenomenon.

In the following table (B) the average duration of the intermediary period (period of rest) is given for both ventricles in the different animals, under various conditions. In making up the averages, those cases which had no relaxation period (*i. e.,* where rigor began in some part of a ventricle immediately after the last part of the ventricle stopped beating) were included as zero. In those few cases where the "period of rigor" overlapped the "period of pulsation" (*i. e.,* where rigor began at the base while the apex was still beating) the period of relaxation was considered as a negative quantity, and the amount of negativity was deducted from the sum total. (Except in fractions of 0.5 round numbers are given; fractions larger than 0.5 were added as 1.0 to the figures.)

TABLE B.

Shows the Average Time of the Period of Relaxation in the Ventricles.

Number and Species of Animal.	Conditions of Experiment.	L. V.	R. V.
26 dogs.	Total of dogs.	28 minutes.	(1) 69 minutes.
20 dogs.	Hearts *in situ*.	23 "	(2) 66 "
14 dogs.	Prolonged etherization.	26 "	59 " (?)
6 dogs.	Short etherization.	24 "	(3) 82 " (?)
3 dogs.	Hearts in Ringer.	23 "	(4) 8 "
3 dogs.	Hearts in 0.9 per cent. NaCl.	48 "	147 "
15 cats.	Total of cats.	16 "	21.5 "
7 cats.	Atropinized.	11 "	19 "
8 cats.	Non-atropinized.	19 "	25 "
3 rabbits.		23 "	(5) 130 "

(1) 25 dogs only. (2) 19 dogs only. (3) 5 dogs only.

(4) In one experiment there was a period of relaxation of 25 minutes' duration; in the other two experiments there was no period of relaxation.

(5) For the right ventricle there was, for two animals, no record at all and on two others the periods of relaxation were very long and the rigor began during the night. In the only animal in which it was ascertained the period of relaxation was about 130 minutes.

Table B shows that the periods of relaxation are in certain respects similar to the periods of pulsation. They are much longer in the right than in the left ventricle; they also seem to be some-

what prolonged by etherization and shortened by atropinization. The influence of the bath is rather peculiar. The Ringer bath has no special influence upon the length of the period in the left and seems rather to shorten it in the right ventricle. The sodium chloride bath on the other hand, seems to prolong greatly the period of rest especially in the right ventricle.

IRRITABILITY OF THE HEART DURING THE PERIOD OF REST.

During the period of rest the ventricles were frequently palpated for the purpose of testing the oncome of rigor. Such a palpation though carried out gently frequently brought out a complete pulsation of the entire ventricle. A review of these observations brought out some interesting results with reference to the irritability of the heart during the period of rest. This question was then studied in six dogs more systematically by means of electrical stimulation. The results are very instructive. Briefly stated they are as follows: At the beginning of the period of rest, stimulation of any part of the ventricle will bring out a contraction of the entire ventricle, and sometimes even of both ventricles. With the advance of time the effect becomes diminished in two directions. In the first place the extent of the contraction becomes gradually lessened. From the contraction of the entire ventricle or a good part of it, the response becomes gradually lessened until the contraction is finally reduced to the point just around the electrode. Before it reaches that minimum, the response comes sometimes in the form of fibrillary contractions. And again, like the spontaneous contractions this occurs oftener in the right than in the left ventricle. When the stimulation by single electric shocks no longer elicits some kind of a response then a tetanizing stimulus might still cause some sort of a contraction, but finally even those stimuli remain without any effect.

Then there is a gradual loss of irritability in a topographical sense. Like the spontaneous contractions the irritability is lost first in the part of the ventricle nearest the base, and remains longest at the very tip of the apex. It was quite a frequent phenomenon to find that the upper part had lost all irritability while the apex was still responding to a stimulus with a fair contrac-

tion. In fact there were instances in which the apex was still beating spontaneously after the upper part of the ventricle had lost all irritability.

We may mention that in one instance when all irritability was completely lost in the left ventricle, circumscript mechanical stimuli applied strictly to the left ventricle brought out complete contractions of the resting but still irritable right ventricle. While this is only a single instance it demonstrates an important principle. It shows that a tissue which has lost all irritability may still be capable of receiving and transmitting stimuli to distant responsive tissues. We can thus understand how for instance a quiescent and unresponsive right auricle might still be capable of receiving stimuli from a source higher up and transmitting them to the ventricles below—a point of contention between the neurogenic and myogenic camps.

THE PERIOD OF RIGOR.

Cardiac rigor as a rule develops slowly. Its very beginning we recognized by a change in the consistency of the tissue. Soon, however, there was also a change in the shape. Strassmann called attention to the shortening of the transverse and long diameters of the heart in rigor. We wish to call attention to the *increase of the antero-posterior diameter* which is at least as striking as the shortening of the transverse diameter and surely more striking than the shortening of the baso-apical diameter. These changes are manifested in a striking way in the development of rigor of the left ventricle. But they are also present and unmistakable in in their manifestations in the right ventricle. At the beginning of our studies—we began with rabbits—we had some misgivings about our capability of judging the phases of rigor in the right ventricle. However, we soon learned that there was little difficulty in recognizing the beginning, progress and maximum of rigor of the right ventricle.

The maximum rigor we have simply judged by the absence of further increase in the consistency as well as in the change in size and shape. When both ventricles are in maximum rigor, the interventricular groove is deeper than normal and the right ven-

tricle is visibly smaller than the left. It looks more like a lateral appendage of the left ventricle.

The observation of the *progress of the rigor* brought out the interesting fact that it also *advances topographically from the base toward the apex of the heart.* The uppermost part of the left ventricle gets into a state of rigor first and appears at the angle formed by the anterior descending ramus, and the ramus circumflexus of the left coronary artery, like a lump raised above the level of the remainder of the heart.

The time consumed in the advancement of rigor is spent not only on an increase of the intensity, but also on the topographical advance toward the apex.

During the topographical march of the rigor it frequently happened that the apex was still quite irritable while the basal portion was in unmistakable rigor. In fact, in a few cases it even happened that the apex or its lowest part was still *beating,* while a portion near the base was already in rigor.

We have not made a single observation in which a region of the heart already in distinct rigor still responded to stimulation. This is of interest considering the statement made concerning skeletal muscles while in rigor.

We made no direct observation on the question of whether in each section of the ventricle the entire thickness of the wall gets into rigor at once, or whether there is also in this respect a certain progressiveness. We are inclined to assume the latter and believe that the rigor begins on the inside and advances toward the outer surface. In support of this assumption we can offer the following observation: when the irritability in any part of the ventricle was on the point of disappearance, *an electrical stimulation sometimes caused a contraction of only the superficial muscle fibers of a circumscribed area.* In these cases apparently the inner muscle fibers had already lost their irritability. Since we now have sufficient reasons to assume that rigor sets in first where the irritability first disappears, it seems that we are justified to suppose that the loss of irritability, as well as the onset of rigor, begins on the inside and ends on the outside surface of the ventricular muscle.

Time of Onset of Rigor.—From what was said above about the

periods of pulsation and relaxation, it is evident that even in the most susceptible parts of the left ventricle rigor does not set in immediately after death. We may now say definitely that under normal conditions there was not a single exception to that rule.

The following is a table of the average time which elapsed between death and the onset of rigor for both ventricles of all the normal animals upon which we have made such observations.

TABLE C.

Showing the Average Time Elapsed Between Death and the Beginning of Rigor.

Number and Species of Animals.	Conditions of Experiment.	L. V.	R. V.
26 dogs.	Total of dogs.	55 minutes.	(1) 115 minutes.
20 "	Hearts *in situ*.	33 "	(2) 96 "
14 "	Prolonged etherization.	39 "	(3) 105 " ?
6 "	Short etherization.	31 "	99 " ?
3 "	Hearts in Ringer.	114 "	138 "
3 "	Hearts in 0.9 per cent. NaCl.	117 "	212 "
15 cats.	Total of cats.	30 "	64 "
7 "	Atropinized.	23 "	55 "
8 "	Non-atropinized.	34 "	78 "
5 rabbits.	Total of rabbits.	56 "	(4) —

(1) 25 dogs. (2) 19 dogs. (3) 13 dogs.

(4) The data on R. V. in rabbits do not permit the giving of exact figures.

The data on the right ventricle were incomplete on certain points. The long duration of the periods made some observations less definite than the observations upon the left ventricle. Some discrepancies in this table have their origin, partly at least, in the fact that in some cases rigor began in one part of the ventricle while another part was still beating. The time during which this took place had to be deducted from the sum total.

The foregoing table (C) shows definitely that the right ventricle in all animals begins to pass into rigor distinctly later than the left ventricle. Even for rabbits, in the experiments of which our data do not permit the giving of exact figures, we can state positively that rigor in the right ventricle begins much later than in the left. The table indicates also, in a general way, that etherization and salt solutions retard, and that atropinization hastens more or less the onset of rigor. The retardation of the onset of rigor by the salt baths is rather marked.

The Growth of Cardiac Rigor.—The following table gives the average time which elapsed between the onset of rigor and the attainment of its maximum.

TABLE D.

Showing the Average Time Elapsing from the Beginning of Rigor to the Attainment of its Maximum.

Number and Species of Animals.	Conditions of Experiment.	L. V.	R. V.
26 dogs.	Total of dogs.	145 minutes.	(1) 143 minutes.
20 "	Hearts *in situ*.	138 "	(2) 153 "
14 "	Prolonged etherization.	121 "	(3) 139 "
6 "	Short etherization.	150 "	184 "
3 "	Hearts in Ringer.	98 "	108 "
3 "	Hearts in 0.9 per cent. NaCl.	251 "	153 "
15 cats	Total of cats.	43 "	(4) —
7 "	Atropinized.	31 "	(4) —
8 "	Non-atropinized.	54 "	(4) —
5 rabbits.	Total number of rabbits.	45 "	(4) —

(1) 25 dogs. (2) 19 dogs. (3) 13 dogs.
(4) Data on right ventricle incomplete.

The figures in Table D bring out some differences between the period of growth of rigor and the two preceding periods. In the first place it appears that the time required for the development of rigor is not so strikingly different between the left and right ventricles as is undoubtedly the case in the periods of pulsation and relaxation. The average time for all the twenty-six dogs—for which there were also complete data on the right ventricle—is, for the period of growth, nearly the same in both ventricles. This, however, is essentially due to the fact that in the *saline experiments the period of growth of rigor is much longer in the left ventricle than in the right.* Furthermore, there appears to be a difference also with regard to the effect of etherization. While the periods of pulsation and relaxation are lengthened by prolonged etherization (*i. e.,* in other words etherization delays the *onset* of rigor), the *growth of it is apparently hastened by prolonged etherization.* Atropinization, on the other hand, appears to hasten both the onset and the growth of rigor. Suspension of the heart in a Ringer solution hastened the growth of rigor (using hearts which remained *in situ* as a standard), while suspension of the heart in a 0.9 per

cent. solution of sodium chloride retarded the growth of rigor considerably.

The growth of rigor—as its onset—progresses, as already stated above, topographically from the base towards the apex, and probably also from the endocardium towards the epicardium. The progress is from section to section, and presumably also from muscle fiber to muscle fiber within each section.

Possibly there is a progression of the onset of rigor even within each individual fiber, that is, every part of an individual fiber may not get into the same state of rigor simultaneously. The time which passes from the beginning of cardiac rigor until its full development is consumed, in part at least, by its topographical progress. There is probably also a growth in the intensity of the rigor within each part. However, we have no experimental facts to help us in an intelligent discussion of these surmises.

DISCUSSION OF THE RESULTS.

According to the last and most extensive work on cardiac rigor, that of Rothberger, the heart very soon after death passes into a state of more or less strong contraction. Rothberger considers this a state of tonic contraction. Other investigators who studied the subject by the same method as Rothberger—the connection of the heart with a manometer—have apparently observed the same condition, and consider it a state of rigor.

Our observations bring out an entirely different result. Except in a single doubtful case, in all our normal (control) animals we did not meet with a single instance in which the heart soon after death was in a state of contraction. After the termination of the life of the animal, in the majority of cases, the beginning of cardiac rigor was preceded by two periods: the period of postmortem cardiac pulsations and the period of complete cardiac relaxation. At any rate our direct observations show that, practically in all cases, rigor was separated from death by a fairly long period of complete relaxation.

The setting in of rigor, or of a continuous tonic contraction soon after death, which was observed by the other experimenters is, we have reason to assume, an artificial phenomenon brought on by the method they employed. The mechanical effect of the distension and the chemical effect of filling up the ventricular

cavity with saline, etc., brings on in the still very irritable heart a tonic contraction which in some cases might indeed hasten the development of rigor. We agree, then, with Rothberger that the distension of the heart after death may cause tonic contraction, but on the basis of our observations we must disagree with him that such a rigor-like tonic contraction occurs after death when the heart remains in normal conditions.

Our observations showed further—to bring this point out separately—that in nearly all animals after exsanguination and respiratory death, the heart continued to beat for a fairly long time. For the left ventricle the average time in rabbits was thirty-two minutes in dogs twenty-seven minutes, and in cats sixteen minutes. The right ventricle was beating in all instances much longer than the left. It must be remembered also that the hearts were exposed to room temperature. In the half dozen experiments on hearts of dogs which were left in baths of salt solutions (room temperature), the pulsation continued in some instances for longer than two hours. This is the more noteworthy as none of the solutions could enter through the coronary arteries.

The literature contains very few statements as to the length of time the heart, when left alone, may continue to beat after death.

MacWilliam speaks of "oscillations which look like systoles and diastoles." But they were infrequent, irregular and occurred as late as two hours after death. These occasional contractions apparently have nothing in common with the pulsations which we have observed to occur regularly for some time after death. Rothberger says that post-mortem pulsations occur frequently in death, after exsanguination, but gives no intimation as to the length of time they may last. He also says that pulsations often accompany the "primary dilatation." From his statements it seems that he has not observed any pulsations during the period of tonic contraction which appears immediately after connecting the heart with the manometer. In one case in which there were some slow pulsations at the beginning of the manometric observations, the connection was made two hours after death. From all these facts it appears that in these experiments *the intraventricular distension causes a continuous systole which thus wipes out the regular pulsation.*

This is a very interesting fact. While in normal life, or soon after death when the circulation of the coronary arteries is re-established, intraventricular pressure, when it acts as a stimulus, can at the utmost start pulsations or effect their character, but can never produce a continuous systole—a tetanus of the heart; we see here that soon after death the heart without a coronary circulation responds to an intraventricular pressure with a tonic contraction—a continuous systole. Furthermore, later, when the irritability of the heart becomes more decreased, distension of the heart without the aid of coronary circulation, does not cause a tonic contraction but is capable of bringing out some rhythmical pulsations. These are significant facts, into a further discussion of which, however, we shall not enter here.

Our observations have shown also—to point out again—that there has been in all animals, with a very few exceptions, an outspoken difference between the left and right ventricles with regard to the length of the periods of pulsation and of relaxation. In all the observations on the three species of animals the right ventricle did beat longer, persevere afterward in a state of complete relaxation and lose its irritability much more slowly than the left ventricle. In other words, in all cases the rigor set in later in the right than in the left ventricle.

For the time taken up by the growth of rigor the difference between the two ventricles is not so striking as it is in the other periods.

From the forensic literature we learn that a great deal of importance used to be attached to a post-mortem finding in which the right ventricle was still in a state of diastole, while the left ventricle was already in rigor. According to our observations such findings could be met with in every case. In fact, in some cases it occurred that the left ventricle was already in fair rigor while the right was still beating fairly rhythmically.

While the fact that rigor sets in later in the right ventricle than in the left is surely not an entirely new observation, no special attention was paid to it in any of the recent studies on the subject. We find statements saying that the heart passes into rigor at this or that time, without defining which part of the heart is meant. In

fact it is in the nature of the method which was employed in most of these studies that in one animal as a rule only one ventricle was studied. But it seems that in some instances the results obtained from both ventricles were not kept separately.

The difference between the two ventricles seems to be based essentially upon greater perseverence of the vitality and irritability of the right ventricle over the left. As to the origin of this difference we wish to call attention to some conditions which possibly serve as causal factors. The right ventricle, as a part of the venous end of the circulation contains, as a rule, soon after death—even when death is caused by bleeding—more blood than the left ventricle. There are good reasons for the assumption that this blood can reach the muscle fibers of the right ventricle and exert some nutritive (or other favorable) influence upon them. Porter[12] and Pratt[13] have shown that nutritive solutions can reach the muscle fibers within the ventricular wall from the cavity of the right ventricle through the foramina Thebesii. Besides, we have seen in our experiments that simple bathing of the heart (with ligated vessels) improves and prolongs the pulsations and the irritability of the heart. This effect can only be brought about by the liquid reaching the muscle fibers through the epicardium. Why could we not assume that the nutritive and stimulating liquid within the ventriclar cavity may also reach the muscle fibres directly through the endocardium? Now, since the right ventricle contains, as a rule, a good deal more blood than the left, the muscular tissue of the right ventricle will be better and longer provided with nutritive and stimulating material than the left—venous blood being at least as good a medium as a Ringer solution. Furthermore, the thinness of the wall of the right ventricle might be another cause of its great vital resistance. In our experiments the favorable influence of the saline baths was generally more pronounced upon the right ventricle than on the left, on account, as we assume, of the lesser bulk of the right ventricular wall, the solutions being able to reach a greater percentage of its constituent muscle fibers than in the wall of the left ventricle. This ought to be true also for the venous blood within the right ventricle. To recapitulate: the greater perseverance of the vital activity and irritability of the right ventricle over the left may be due to (1) that the blood within the cavity of the right ventricle is capable of reaching the muscle fibers of the wall through the foramina Thebesii and also directly through the endocardium; (2) that on account of the thinness of the wall a greater percentage of the fibers can be reached by the nutritive medium than in the bulky wall of the left ventricle; and (3) the right ventricle, as a rule, contains more blood after death than the left ventricle.

Our observations revealed the interesting fact that the various post-mortem changes which precede complete rigor progress topographically from the base towards the apex. The spontaneous

[12] Porter, *American Jour. of Physiol.*, 1898, i, 71.
[13] Pratt, *idem.*, 1898, i, 86.

pulsations do not cease in all parts of the ventricle at once, but stop at the uppermost part first and in the tip of the apex last. The same topographical succession takes place in the disappearance of irritability of the ventricles during the period of relaxation. Finally, rigor appears first in the upper part of the ventricle and spreads gradually to the apex. As we have stated before, we have reason to assume that the rigor wanders also from the inner surface towards the epicardium. This wandering of post-mortem changes is confined to each ventricle separately and it sometimes happened that the apex of the left ventricle was already in rigor while one part of the right ventricle was still beating.

L. Krehl[14] and F. R. Fuchs[15] have established, as stated before, that some time after death, liquids injected into the ventricular cavities cannot pass into the auricles. This is due, according to Fuchs, to the onset of rigor in the ventricles. Fuchs considers the impassability of liquids through the venous ostia as an early phenomenon of rigor and states that it may occur before the ventricles show any other signs of rigor. Connecting these observations with our own, we might assume that rigor begins with the innermost muscle fibers at the uppermost part of the ventricle and around the venous ostia, thus producing early a stenosis of the corresponding orifices which sometimes might be capable of preventing the passage of liquid from the ventricle into the corresponding auricle. This is perhaps the cause of the not infrequent occurrence that the right ventricle while already in full rigor still contains some blood.

Strassmann[16] has called attention to the early appearance of cardiac rigor. Fuchs[17] has pointed out that rigor appears in the heart before it appears in the skeletal muscles. Both speak of the heart in general, which is inadequate, inasmuch as the rigor of the skeletal muscles might be already advanced before it had made its appearance in the right ventricle of the heart. However, the statement of Fuchs seems to be true with regard to the onset of rigor in the left ventricle. In this regard we may say in general

[14] L. Krehl, *loc. cit.*
[15] F. R. Fuchs, *loc. cit.*
[16] Strassmann, *loc. cit.*, 1889.
[17] Fuchs, *loc. cit.*

that rigor was noticed in a part of the left ventricle before it was noticed in any group of skeletal muscles. That this is not due exclusively to the constant activity of the heart, as Fuchs assumes, is evident from the fact that rigor of the right ventricle, which works as constantly as the left, sets in rather late.

Finally, we might recall the fact that in our observations *ether delayed the onset and hastened the growth of cardiac rigor, while atropin accelerated both phases.*

[NOTE.—Summary of Part I will follow with Part II of this paper.]

THE RELATION OF THE WEIGHT OF THE CONTENTS OF STOMACH AND CECUM TO THE BODY-WEIGHT IN RABBITS.[1]

By DON R. JOSEPH.

(*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*)

In much of our experimental work upon mammals, the dosage of the various drugs used is regulated according to the size of the animal—a given amount being allowed per kilo of body-weight. In the rabbit this is a source of considerable error on account of the large size of the stomach and cecum. After a full feeding, when these organs have become well filled, the weight of their contents bears a relation to the total body-weight which is quite out of proportion to this same relation in other animals.

In order to determine just how great an error would obtain from an inclusion of these contents in the body-weight of a living rabbit, I have removed and weighed the material found in the stomach and cecum in the case of fifty male and fifty female rabbits which were used in the laboratory for other purposes. In each case a record was kept of the sex, body-weight, weight of the stomach, weight of the stomach contents, weight of the cecum, weight of the cecum contents, and the number of hours the weight was taken after feeding.

In removing the contents of the cecum an opening was made near the ileo-colic end, and by compression from the outside, everything was removed which could readily be expelled. Not more than three to five grams were ever left. None of the rabbits in my series were selected or fed beforehand in order to raise the percentage. Every rabbit that was killed in the laboratory was used until one hundred weights were obtained. Some had been

[1] Received for publication September 25, 1908

fed a few minutes before being weighed and used, others had not been fed for five, twelve, seventeen or twenty-three hours.

An examination of the tables below shows the following points concerning the one hundred animals examined:

1. The stomach- and cecum-contents in male rabbits are equal to an average of 10.4 per cent. of the whole body-weight. In female rabbits to 11.4 per cent. of the body-weight.

2. The lowest percentage found between stomach- and cecum-contents and the body-weight in males was 4.74 per cent. in females 6.80 per cent.

3. The highest percentage found in males was 19.32 per cent.; in females 18.04 per cent.

4. The average weight of stomach-contents in males having an average body-weight of 1665 grams is 94 grams. In females with an average body-weight of 1674 grams this average is 106 grams.

5. The average weight of cecum-contents in males is 80 grams; in females 79 grams.

6. Small rabbits have relatively larger stomach- and cecum-contents than heavier rabbits. In the table of averages for female rabbits (Table III, Columns 4 and 5), this is easily seen. The male rabbits (Table II) do not show it so markedly.

The amount of material contained in the stomach and cecum varies greatly in different rabbits, and also in the same rabbit at different times. It is manifestly impossible to lay down a rule which will hold in the case of every rabbit. However, since the inclusion of these contents in the body-weight will invariably cause a considerable error, it is possible to formulate a rule which will reduce the error to a minimum. For practical working purposes (and for the sake of simplicity) it is safe, judging from these tables, to allow ten per cent. of the body weight of any rabbit for the contents of stomach and cecum.

This rule may be stated in another way, although it is then somewhat more complicated. It is as follows: allow 125 grams for the first kilo of body-weight and 25 grams additional for each 300 grams of body-weight above one kilo.

Grober[2] has recently made a series of similar observations in con-

[2] Grober, "Untersuchungen zur Arbeitshypertrophie des Herzens." *Deutsches Arch. f. klin. Med.*, 1907, xci, 504.

TABLE I.

Sex.	Average Body-Weight. Grams.	Average Weight of Contents of Stomach. Grams.	Average Weight of Contents of Cecum. Grams.	Average Total Weight of Contents of Stomach and Cecum. Grams.	Corrected Body-weight. Grams.	Average Percentage of the Stomach- and Cecum-contents to the Body-weight. Per Cent.	Lowest Percentage Found Between Stomach- and Cecum-contents and the Body-weight. Per Cent.	Highest Percentage found Between Stomach- and Cecum-contents and the Body-weight. Per Cent.
Male	1,665	94	80	174	1,491	10.41	4.74	19.32
Female	1,674	106	79	185	1,489	11.36	6.80	18.04

TABLE II.

Male Rabbits. Table of Averages.

Body-weight.	Average Weight of Stomach-contents. Grams.	Average Weight of Cecum-contents. Grams.	Average Weight of Stomach- and Cecum-contents. Grams.	Average Percentage of Stomach- and Cecum-contents to the Body-Weight. Per Cent.
1,000–1,299	64.0	66	128	10.80
1,300–1,499	85	66	151	10.87
1,500–1,599	89	65	154	9.76
1,600–1,899	93	96	189	11.04
1,900–2,199	112	85	197	9.70
2,200–2,899	133	105	238	9.37

TABLE III.

Female Rabbits. Table of Averages.

Body Weight.	Average Weight of Stomach-contents. Grams.	Average Weight of Cecum-contents. Grams.	Average Weight of Cecum- and Stomach-contents. Grams.	Average Percentage of Stomach- and Cecum-contents to the Body Weight. Per Cent.
800–899	65	83	148	18.04
1000–1299	96	64	158	12.82
1300–1399	84	77	155	10.81
1400–1699	110	83	193	12.01
1700–1999	106	78	184	9.85
2000–2399	128	85	213	9.65

nection with some work on hypertrophy of the heart, in which he tied off, removed, weighed and included, the weights of the stomach and cecum with that of their contents. He considers that the inclusion of the weight of the stomach and cecum with the weight of their contents will not be the source of any appreciable error, since there is such a wide variation in the weights of the contents

of stomach and cecum in different rabbits. However, according to my results, the average weight of the empty stomach is 26 grams, and of the empty cecum 36 grams, a total of 62 grams. It is easily seen that the addition of 62 grams to the weight of the contents of stomach and cecum of every rabbit (especially in the case of those whose stomach and cecum were not well filled) would materially change the percentage relationship between these contents and the body-weight.

Again, Grober's[3] tame rabbits have an average of 276 grams weight of stomach and cecum plus contents, per kilo of body-weight, or 27.6 per cent. of the body-weight allowed for in these organs. If we suppose that the average weight of the stomach and cecum in his rabbits was the same as my own (62 grams), there would still remain in his rabbits 214 grams per kilo of animal for contents of stomach and cecum, *i. e.*, 21.4 per cent. of the body-weight. Now my rabbits show an average of 103.5 grams of stomach- and cecum-contents, per kilo of body-weight (*i. e.*, 10.35 per cent. of the body-weight), or about 110 grams of stomach- and cecum-contents less than Grober found in his rabbits.

A comparison of Grober's tables with mine shows that for rabbits of any given weight, the heart weight is approximately the same, so that the variation between our percentages for stomach- and cecum contents must be due to both the inclusion of stomach and cecum with the weight of their contents and also to the presence of a larger amount of food material in the stomach and cecum of his rabbits than in those examined by me. I cannot acount for the large figures obtained by Grober unless his rabbits were fed not long before being used or else were fed more heavily than the rabbits at my disposal.

[3] Grober, in his article, does not give direct figures for the weight of the organs and their contents. He gives only the ratio between the heart-weight and body-weight before and after removal of the stomach and cecum with their contents. The number of grams of heart tissue per kilo of animal (*i. e.*, the ratio) before removing the stomach and cecum he finds to be 2.36; after removal, 3.26. In other words the removal of these two organs with their contents raised the ratio of the heart-weight to the body-weight, 0.9 gm. per kilo of animal. From these figures we find that the weight of the stomach and cecum plus their contents must have been 276 grams per kilo of body-weight.

In the paper published recently[4] on the ratio between the heart-weight and body-weight in various animals, I made the following statement in commenting on the very low ratio between the heart-weight and body-weight in rabbits: "A ratio derived from rabbits with the weight of the contents of stomach and cecum excluded, would undoubtedly be nearer that found in the other animals (dogs, approximately, 7.5, cats 4.5, guinea-pigs 4.0, rabbits 2.6)."

While making the observations given in the present paper, the heart-weight was taken in each rabbit. I find that the ratio of the heart-weight to the body-weight was increased about .31 gram per kilo of animal by the removal of the stomach- and cecum-contents, *i. e.*, 50 male rabbits with an average body-weight of 1665 grams gave a ratio of 2.60 before removal and 2.91 after removal. Fifty female rabbits with an average body-weight of 1674 grams gave a ratio of 2.55 before and 2.88 after removal of the contents of stomach and cecum. This calculation shows that in rabbits, even after removal of the contents of stomach and cecum, the ratio between heart-weight and body-weight is considerably less than in dogs, cats, or guinea-pigs.

[4] *Jour. of Exper. Med.*, 1908, x, 521.

THE BORDET–GENGOU BACILLUS OF PERTUSSIS.[1]

By MARTHA WOLLSTEIN, M.D.

(*From the Rockefeller Institute for Medical Research, New York.*)

PLATE I.

BACTERIOLOGY.

Since Jochmann and Krause (1) described the influenza-like *Bacillus pertussis, Eppendorf,* it has generally been admitted by the majority of investigators on the subject that influenza-like bacilli are present in all, or nearly all, of the specimens of sputum studied from persons suffering from whooping cough. A new element was introduced by Bordet and Gengou (2), in 1906, when they described a hitherto unknown bacillus as the specific etiological factor in pertussis, basing the claim of specificity upon the facts that the bacillus is found in overwhelming numbers and in an almost pure state in the sputum during the early days of the disease, that is, when the whoop has but just appeared, and that the phenomenon of complement deviation (3) is obtained with a salt solution suspension of the bacillus mixed with a measured quantity of serum from a convalescent patient and a suitable hæmolytic system. Bordet and Gengou found that the influenza bacillus, in similar mixtures, gave no complement binding reaction. The following year these observers (4) published further details on the biology of their bacillus, on the irregularity of its agglutination reactions, and on the not very favorable results of protection experiments with the serum of an immunized horse. The *Microbe de la Coqucluche* is short, poled, ovoid, a trifle larger than *B. influenzæ*, not motile, very regular in size and shape, arranged singly within groups, occasionally in pairs, either end to end or parallel. It is negative to Gram's stain, but colors especially well with toluidine-methylene blue. While the poles stain deeply, a pale or unstained area remains in the center of the

[1] Received for publication September 20, 1908.

small ovoid. This characteristic is very marked and very regularly present in young cultures. Bordet and Gengou (2) devised a glycerine-potato-blood-agar medium for the isolation of the bacillus.

During the past winter and spring cases of pertussis were studied at the Babies' Hospital and at the Children's Hospital on Randall's Island, through the courtesy of the Medical Board and the Commissioner of Charities. To these gentlemen I wish to express my obligation.

Of the twenty cases that came under my observation, one was in the seventh week of the disease, one in the sixth, five in the third, five in the second, five at the end of the first week, one on the first day of the whoop, and one was first seen two days before she began to whoop, the cough having lasted one week. The Bordet-Gengou bacillus was isolated from five cases: one in the second week, two in the first week, one on the first day and one two days before the whoop began. The influenza bacillus was isolated from ten cases, including two which showed the Bordet-Gengou organism as well. In five others Gram negative bacilli, poled and intra-cellular, were found in smears, but attempts at isolation were unsuccessful; while in two cases, in the third and sixth weeks respectively, repeated examinations failed to find any other bacteria than cocci and Gram positive bacilli. Dr. Flexner had obtained a culture from Professor Bordet which was used as a control throughout this work, as was another culture of the original Pfeiffer influenza bacillus also obtained from Professor Bordet.

As much sputum as possible was secured from a case, but that obtained at the end of a paroxysm of coughing only was found of use. The sputum was hurried to the laboratory, and smears and plate cultures prepared at once from the white, bronchial flakes, both before and after washing in salt solution. The films were stained by Gram's method and with toluidine-methylene blue.

In the case of the child who came under observation before the whoop had developed, the films showed many intracellular and free bacilli. On the plates spread with a thin flake of the sputum very small and delicate colonies were visible in twenty-four hours, many new ones appearing on the following day. Impression preparations showed that there were only Gram negative bacilli present

but that they were not all alike, differences in regularity of size and arrangement, not so marked as to exclude the possibility that they were due to the age of the respective colonies, some being twenty-four hours older than others, were plainly seen. In tubes inoculated from these colonies, it became apparent that only *B. influenzæ* was growing, and in going over the original plates again on the fourth day, larger colonies were transplanted which proved to agree with the control Bordet-Gengou culture. Thus at this early stage of the disease, before the spasmodic stage had been established, both the Bordet-Gengou bacillus and *B. influenzæ* were present in the bronchial secretion.

The most characteristic specimen was obtained from sputum on the first day of the spasmodic stage. In the smears small, poled, Gram negative bacilli were present in enormous numbers and almost in pure cultures, lying in large groups made up of single members. Many leucocytes contained from six to twenty bacilli. The only other bacteria present were biscuit-shaped diplococci, also negative to Gram's stain. Potato-blood-agar plates were spread with flakes of washed sputum and in twenty-four hours an almost invisible, delicate growth was apparent besides the large colonies of *M. catarrhalis*. Impression preparations showed that the Gram negative poled bacilli made up this delicate film. Twenty-four hours later the colonies could be plainly seen and studied, and pure cultures on potato-agar slants were readily obtained. These grew on all bood media but delicately, failing to acquire the more vigorous appearance of the control Bordet-Gengou culture which had been grown artificially for many months. Sputum films still showed large numbers of the poled bacilli, but pneumococci as well. Before another culture could be isolated the child died. At autopsy plates were inoculated from the trachea, bronchi and lung substance, which showed large areas of broncho-pneumonia, the anatomical diagnosis reading: general bronchitis; broncho-pneumonia; hepatic and renal congestion.

Films made from the trachea, bronchi and lungs at autopsy showed many characteristic Gram negative bacilli and many lance-shaped Gram positive diplococci. On the plates only two kinds of bacteria grew, pneumococci and Gram negative bacilli, presenting

in the plates from the lungs all the characteristics of the control Bordet-Gengou culture. The influenza bacillus was isolated from the trachea. The bacillus grown from the sputum during life and from the trachea at autopsy did not grow on serum agar without hæmoglobin, while on potato-blood-agar it grew with a delicate film made up of single colonies, the medium becoming very dark in color. The culture from the lung substance grew on serum-agar and left the blood-agar unchanged in color. Bordet and Gengou call attention to this change in the color of the blood medium and give it as their opinion that a sure way to differentiate between these two varieties of bacilli is by means of blood-agar cultures.

In this child, A. S., then, the influenza bacillus was present in the sputum on the first day of the whoop, and it shows that this organism is present not only late, but also early in pertussis sputum. The case examined before the establishment of the whoop confirms this view. Davis (10) also found the bacilli present in two children before the whoop began. Both of my early cases also show how readily the Bordet-Gengou bacillus, though present in apparently overwhelming numbers, may be missed in cultures because the influenza bacillus grows so much more readily and quickly on all blood-agar media. They also bring out the fact that in sputum it is not possible to differentiate between the two organisms morphologically. In culture the more irregular forms of *B. influenzæ* soon make themselves apparent.

Microscopic examination of sections from the lungs of the case A. S. showed broncho-pneumonia with marked purulent infiltration, and very little fibrin formation. Number of alveoli showed necrosis of their exudate, the necrotic areas being outlined by masses of Gram negative bacilli, presumably the Bordet-Gengou organism. This condition is well shown in Figs. 1 and 2. There was also a very general bronchitis and peribronchitis, involving large and small tubes alike. The capillaries in the alveolar walls were uniformly and markedly congested, but very few bacilli were found in any blood vessels.

Throughout this work rabbit's blood was used for all the blood media required. Both pigeons and guinea-pigs were tried, but they possessed no advantage. On the potato-blood-agar plates the col-

onies are, in fresh cases, barely visible in twenty-four hours; in forty-eight hours they are quite plain, discreet, small and greyish; they increase in thickness rather than in size. On slanted tubes of the potato-blood-agar medium the growth is, after the first generation, very characteristic, being dense, grey, glistening and much more vigorous than that of the influenza bacillus, while on ordinary blood-agar the growth is a thin, delicate film of single colonies, easily mistaken for those of *B. influenzæ*. On human or rabbit serum-agar without blood the Bordet-Gengou bacillus grows well, with a moist, greyish white film, usually delayed forty-eight or seventy-two hours in its appearance. No growth occurs on plain agar or in plain broth or litmus milk. On bouillon mixed with human or rabbit serum, whether or not blood be present, the bacillus grows vigorously, forming a large viscid precipitate but no surface film. The reaction of the broth remains unchanged. The addition of glycerine to the serum broth made no difference in the growth or longevity of the bacillus. It remained viable two months in human ascitic broth, and at the end of that time was still regular in appearance and small in size, lying singly and in groups, a few pairs and chains of four elements being found, the individuals taking the stain well. As Bordet and Gengou pointed out, the bouillon cultures grow better when the tubes are kept in a slanted position, because the bacilli are very strictly aerobic. In lactose, saccharase, dextrose and mannite-litmus-serum water, the bacillus grew, but caused no change in the medium.

While growth is most rapid at a temperature of 35° to 37° C., it proceeds well at the ordinary room temperature, more slowly at 5° to 10° C. When kept at 0.5° C. for six days cultures grew vigorously when removed to the thermostat, and smears made at the time of removal from the ice chest showed that proliferation had gone on at 0.5° C. Blood-agar cultures kept in the thermostat were still alive after sixty-two days, although the medium had become shrunken to less than half the original mass and was very brown in color. In the ice chest at 4° to 10° C., the cultures were dead after fifty-three days. Sub-cultures made from the old tubes gave a growth which was more dry than that from recent ones, and the individual colonies were larger, more distinct, with less tendency

to become confluent even at the lower, moist end of the slanted medium.

Films made from potato-blood-agar cultures of the bacillus on the fiftieth day of its existence at 4° C. showed great variations in the dimensions and staining power of the individuals. All were small, but many had become swollen to an almost spheroidal shape, taking the stain very badly or not at all. The contrast between these and the more deeply staining bacilli of more normal size was very marked; in the latter the entire organism was colored uniformly, no pale center and darker ends being apparent. Films prepared from a culture which had been in the thermostat sixty days showed few bacilli, but these still stained well, a few pale ones being seen. No large swollen forms were noted.

When heated at 56° C., for thirty minutes, the bacilli are killed, as Bordet demonstrated. Autolysis is not marked in such heated cultures, and the addition of toluol to heated or unheated suspensions does not hasten autolysis to any appreciable degree. It occurs more rapidly and extensively at low temperatures (4° to 10° C.) than at higher ones (20° C., 35° C., 56° C.).

ANIMAL INOCULATIONS.

Monkeys, rabbits, guinea-pigs, dogs and cats were inoculated with cultures.

Monkeys.—A suspension of a forty-eight hour old culture was injected directly into the trachea, which had been exposed by an incision. The wound healed and the monkeys remained perfectly well.

A suspension of two forty-eight hour old cultures was blown into the nostrils and mouth by means of a metal atomizer. Only five cubic centimeters of fluid were used and the monkey inhaled all of the very turbid mixture. The procedure was repeated twice, but without result. Neither cough nor snuffles had developed three months afterward. This experiment was repeated according to Fraenkel's method (14), but again with negative result.

Cats.—A suspension of a forty-eight hour culture was sprayed into the nose and mouth. The animal showed no symptoms of any kind.

Cultures suspended in milk were fed to the animal on eleven successive days. No effect was noted during a period of five weeks.

Dogs.—Bacilli suspended in salt solution were sprayed into the nose and mouth of a young dog. After seven days snuffles developed, followed in two days by suffusion of the eyes and three days later by sneezing and coughing; but no whoop appeared, and the symptoms began to subside in two days more. The dog was chloroformed fifteen days after the inhalations had been begun, and the lungs showed an extensive general bronchitis involving even the smallest bronchioles; there was no pneumonia. Cultures from the heart's blood gave no growth, but from the mucus of the bronchi pure cultures of the distemper bacillus described by Galli-Valerio (5) and by Babes and Barzanesco (6) were obtained. This organism is small, poled and negative to Gram's stain, and in smears prepared directly from the mucus at the autopsy gave rise to the impression that the Bordet-Gengou bacillus had been recovered. Cultures disproved this. Although the dog had appeared to be healthy, the distemper-causing bacillus must have been present in his upper respiratory tract before the experiment began, and it rapidly overgrew the more delicate bacillus of Bordet-Gengou. This dog's serum gave only negative agglutination reactions with the Bordet-Gengou bacillus, and no complement deviation was obtained when it was tested in suitable mixtures.

The above experiment was repeated. Seven days after the inhalations were begun snuffles and sneezing were noticed. It was decided to let the animal live in order to watch the progress of the symptoms. On the ninth day the dog was very ill, sneezed almost continuously, and was too weak to stand. Bloody diarrhœa developed and emaciation progressed rapidly, until the animal died on the nineteenth day. The symptoms were those of distemper and again the bacillus of Galli-Vallerio was found in cultures made from the pneumonic areas and from the bronchial mucus. The heart's blood gave no growth. It is evident that in this animal also the inhalations caused an irritation which paved the way for the development of the previously present bacillus of distemper. The animal had been in the building a short time before the experiment began, and had been alone in his cage.

A normal dog was killed and cultures were made from the lungs. They remained sterile.

Guinea-pigs.—Subcutaneous and intraperitoneal inoculations of living cultures had no effect when used in doses of half to one solid culture.

Rabbits.—Only large doses of two solid cultures, when injected into the peritoneal cavity or into the circulation through the ear vein caused death. Animals dying in twenty to twenty-four hours after intra-peritoneal inoculation showed the following condition at autopsy: the peritoneum was covered with numerous smaller and larger hemorrhages, and about five cubic centimeters of bloody serous fluid were present; the point of inoculation was surrounded by a large hemorrhagic area and occasionally a small flake of fibrin. The omentum was rolled up and hemorrhagic. In some instances small, soft clots of blood were found on the surface of the omentum, liver and intestines. The lungs were pink and well aerated, and only a few drops of bloody serous fluid were present in the pleural cavities. Many leucocytes were found in films prepared from the peritoneal fluid and serous surfaces; these cells contained many bacilli, the majority of which stained badly; there were very few free bacilli. Cultures from the peritoneum remained sterile, while those inoculated from the heart's blood showed a pure culture of the inoculated bacillus, but not a profuse one. Large doses of killed cultures injected into the ear vein of a rabbit killed as rapidly as living bacilli did. Small doses had no effect.

Macewen (7) reports one successful experiment by feeding a cat for seven days with the sputum and vomit of pertussis patients not suffering from broncho-pneumonia. After fourteen to seventeen days the cat became languid, and after one month it developed a choky cough followed by vomiting. Some time later a spasmodic cough with a typical and characteristic whoop appeared. Macewen made no bacteriological examinations.

In an attempt to repeat this experiment, large amounts of sputum from two cases of pertussis in the sputum of which the Bordet-Gengou bacillus had been demonstrated were fed to a healthy, large cat. Very little vomit was mixed with the sputum, however, because these children vomited rarely. On the seventh day the cat began

to sneeze, and continued to do so for three days; she also became hoarse. Then the symptoms subsided, and, although kept under observation for six weeks, the cat remained perfectly well, no cough developing at any time. She was then bled to death, and her serum gave no complement binding reaction in any dilution with Bordet-Gengou or influenza bacillus antigen. A second cat was fed for seven days on large amounts of sputum from a child with pertussis. The entire twenty-four hour output was collected for the purpose from the second to the ninth day of the spasmodic stage. This sputum contained enormous numbers of Bordet-Gengou bacilli and of *B. influenzæ* as well. This cat developed neither cough nor snuffles during the four weeks she was kept under observation. For control, a cat was fed on large doses of cultures of the Bordet-Gengou bacillus, also without causing symptoms of disease.

SERUM REACTIONS.

Agglutination.—Rabbits were immunized with progressively increasing doses of living bacilli injected into the peritoneal cavity, and others by inoculation into the ear vein. The highest agglutination reaction was obtained with the serum of animals immunized by means of the intraperitoneal method. Two series of animals were inoculated, one with the Bordet-Gengou organism and the other with *B. influenzæ*. The agglutination reactions in every instance were sharp and distinct from each other, that is, the Bordet-Gengou bacillus agglutinated as high as one to eight hundred in its homologous serum and remained quite unaffected in the serum of a rabbit immunized with *B. influenzæ*, which agglutinated its own organism in dilutions of one to four hundred. Normal rabbit serum does not agglutinate the Bordet-Gengou bacillus in dilutions higher than one to twenty or thirty.

The blood serum of children with pertussis agglutinated the Bordet-Gengou organism very irregularly, as Bordet has shown. During the first week of the disease only one of the children possessed serum showing any agglutination reactions, and that in only one to forty. In the second week also one child yielded serum giving the highest agglutination which occurred during the six weeks she remained under observation; it was one to two hundred.

B. influenzæ clumped in the same serum in a dilution of one to one hundred. In the third week no agglutination reaction higher than one to one hundred was observed, and the majority were only one to fifty. In the serum of three children the influenza bacillus agglutinated in higher dilutions than the Bordet-Gengou bacillus did. In the fourth, fifth and sixth weeks the agglutinations were not higher than one to fifty except in two children, where it reached a dilution of one to one hundred and one to four hundred respectively. In the former case the agglutination of *B. influenzæ* ran parallel with that of the Bordet-Gengou bacillus. The serum from two cases gave no agglutination with any strain of either bacillus during four weeks observation.

Opsonic Index.—The opsonic index was determined in four children, two during the second week, one during the fourth week and one during the seventh week after the onset of the cough. While the index of the Bordet-Gengou bacillus was low compared with the normal control, and lower than that of the influenza bacillus, heating the serum to 60° C. for thirty minutes had no effect on the opsonizing power, the index falling only 0.04 to 0.2 in any case. As the effect of heating the serum was without influence in the case of *B. influenzæ* as in that of the Bordet-Gengou bacillus, it follows that one was as thermostabile as the other. Therefore neither could be looked upon as more positively an immune opsonin than the other, and the determination of the opsonic index would seem to add nothing to the evidence of specificity for one bacillus more than for the other.

Complement Deviation.—In making complement deviation tests with the sera from patients and from immunized rabbits, three varieties of antigen were employed: salt solution suspensions of growth on solid culture media, as used by Bordet and Gengou; extracts of the bacilli made by suspending the growth of three blood-agar slants in five cubic centimeters of salt solution and shaking twenty-four hours in the thermostat; and extracts from the lungs of the case which came to autopsy, prepared according to Wassermann and Meier's (8) method. The bacilli were always washed in salt solution in order to remove any adherent rabbit blood or serum elements, before they were used as antigen, either in the forms of suspensions or of extracts.

Serum from nine patients was tested, at various times from the first to the ninth week[2] of the disease, when the whoop had practically ceased. Three cases were examined more than once. In no instance did complement binding occur in mixtures containing 0.3 to 0.05 cubic centimeter of the serum, 0.2 to 0.05 cubic centimeter of antigen, 0.1 of fresh guinea-pig serum as complement, 0.05 cubic centimeter of washed sheep's corpuscles and 0.005 cubic centimeter of anti-sheep immune rabbit's serum. All these varieties of antigen were tried with these sera, but no results pointing to the presence of anti-body capable of uniting with the antigen in the presence of complement were obtained in any instance. Tests with influenza bacillus antigen were equally negative in their results.

With the serum from immunized rabbits the results were quite different and very striking. Thus when immunized with the Bordet-Gengou bacillus, either with a strain isolated from a child's sputum or that brought from Bordet's laboratory, the serum bound complement in the presence of a Bordet-Gengou bacillus antigen and not with influenza antigen, or at least not in the same dilutions. The influenza immune serum bound complement with an influenza antigen and not with the Bordet-Gengou antigen. Alcoholic extracts of guinea-pig heart and liver gave results identical with those obtained with the Bordet-Gengou or influenze antigens, the heart extract being more active than that of the liver. This result tends to confirm the notion of the lipoid nature of the antigens giving the deviation reaction (Porges and Meier) (9).

The lungs from the case which came to autopsy were extracted according to the method of Wassermann and Meier (8), and gave successful complement deviation tests with rabbit's serum but not with sera from the children. It is interesting to note that positive reactions were obtained with the serum of rabbits immunized with the Bordet-Gengou bacillus as well as with that from rabbits made immune to *B. influenzæ*, a fact which can be accounted for by the presence of both varieties of bacilli in the lungs in this case. Unfortunately, however, the extract from a lung which was the seat

[2] Two during first week; one on tenth day; two in third week; two in fourth week; one in fifth week; one in sixth week; one in seventh week; two in ninth week.

of empyema and carnification, and from which only streptococci and no bacilli had been isolated, gave similar reactions with rabbit's serum. The complement binding was evidently cumulative and not specific in character.

Meier (11) reported that extracts made from the lungs of three children dying during an attack of pertussis added to the serum obtained from four persons suffering from the disease gave binding of complement, while extracts from the liver and spleen gave little or no result. Controls made with extracts from lungs of children who died of measles gave no binding reaction at all. The strength of the dilutions used is not stated. Meier made no bacteriological examinations. I am unable to explain the discrepancy between his results and my own.

Klimenko (12) found the Bordet-Gengou bacillus in five cases examined during the first and second weeks of the spasmodic stage and also in the heart and lungs of a child who died in the third week of pertussis. He claims to have produced a barking cough in monkeys and young dogs[3] by means of pure cultures isolated from one of his cases. Moreover a healthy monkey, remaining in the cage with another monkey who had been infected through the upper air passages, acquired the cough on the third day. The incubation stage appears very short for true pertussis, which, in children, lasts from seven to fourteen days (Holt). On the other hand, the cat in Dr. Macewen's experiment exhibited an unusually long incubation period, as the cough began one month after the feeding with sputum and vomit.

Arnheim (13) examined sputum from twenty cases of pertussis. and isolated the Bordet-Gengou bacillus from six but failed to find it in five autopsies on children dying during an attack of the disease. Agglutination reactions were positive in dilutions of one to fifty in sixteen convalescent cases, and complement deviation was demonstrated in the serum from six of twelve cases examined. The

[3] Klimenko's full report (*Cent. f. Bakt.*, Orig., 1908, xlviii, 64) appeared after this article had gone to press. The fact that his cultures grew on all ordinary laboratory media and that he has completely ignored distemper among his series of forty-eight young dogs in which he claims to have produced pertussis by inhalation experiments seems to invalidate at least that part of his work completely.

dilutions used are not stated. Attempts to give pertussis to monkeys and dogs were unsuccessful. On the other hand Fraenkel (14) allowed two monkeys to inhale a thick cloud of Bordet-Gengou bacilli for fifteen minutes, with the result that the animals developed a short, paroxysmal, barking cough in five to six days. This disappeared after eight or ten days. Fraenkel found the bacillus in the sputum of eight cases of pertussis, and failed to find it in thirty others. He found that agglutination reactions were irregular and not constant, while positive complement deviation was obtained only once in five sera examined. The most interesting point in Fraenkel's work, which came to my attention after this paper was completed is, however, the fact that he was able to find the Bordet-Gengou bacillus in small numbers in two cases not affected with pertussis, but suspected of being tuberculous. This is the first time that this bacillus has been observed in the sputum of any disease other than pertussis. It has not, thus far, been found in normal persons.

SUMMARY.

The bacillus of Bordet and Gengou is present in the sputum in early cases of pertussis, and in the lungs at autopsy in fatal cases of the disease. After the second week it is not present in the sputum in sufficiently large numbers to be readily isolated. The influenza bacillus is found at as early a stage of pertussis as is the Bordet-Gengou bacillus, and it persists in the sputum for a longer time. The agglutinins in the blood of pertussis patients are not more regular and not always higher for the Bordet-Gengou bacillus than for *B. influenzæ*. The two organisms are culturally distinct and their action on laboratory animals different. Complement deviation tests with the serum of immunized rabbits show a further difference in the immune bodies produced by the two varieties of bacilli. The negative results of the complement deviation tests with the patients' serum in this study, compared with the positive results of similar tests made by Bordet and Gengou, I am not able to account for.

This study has contributed support to the view that the Bordet-Gengou bacillus is the possible cause of pertussis, but it has not produced any distinctively new evidence of this relationship beyond

the proof of the wide dissemination of the peculiar bacillus in nature and its occurrence in pertussis. A study of bronchial secretions in other acute diseases of the respiratory tract for the bacillus has not yet been extensively made and is called for. I shall hope to report on this phase of the subject at another time.

BIBLIOGRAPHY.

1. Jochmann and Krause, *Zeit. für Hygiene,* 1901, xxxvi, 193.
2. Bordet and Gengou, *Annales de l'Inst. Pasteur,* 1906, xx, 731.
3. Bordet and Gengou, *idem.,* 1901, xv, 289.
4. Bordet and Gengou, *idem.,* 1907, xxi, 721.
5. Galli-Vallerio, *Cent. für Bakt.,* Abt. 1, 1896, xix, 694; *Ref.,* 1908, xli, 563.
6. Babes and Barzanesco, *Annales de l'Inst. de Path. et de Bakt. de Bukarest,* 1892–3, v, 431.
7. Macewen, *British Medical Journal,* 1908, i, 146.
8. Wassermann and Meier, *Deut. med. Woch.,* 1907, xxxiii, 1287.
9. Porges and Meier, *Berl. klin. Woch.,* 1907, xliv, 1601 (quoted by Wassermann).
10. Davis, *Journal of Infectious Diseases,* 1906, iii, 1.
11. Meier, *Deut. med. Woch.,* 1907, xxxiii, 1558.
12. Klimenko, *Cent. für Bakt., Orig.,* 1908, xlvi, 218.
13. Arnheim, *Berl. klin. Woch.,* 1908, xlv, 1453.
14. Fraenkel, C., *Münch. med. Woch.,* 1908, lv, 1683.

EXPLANATION OF PLATE I.

FIG. 1. Pertussis broncho-pneumonia, showing necrosis of intra-alveolar exudate and masses of Gram negative bacilli outlining the necrotic areas.

FIG. 2. The same, more highly magnified.

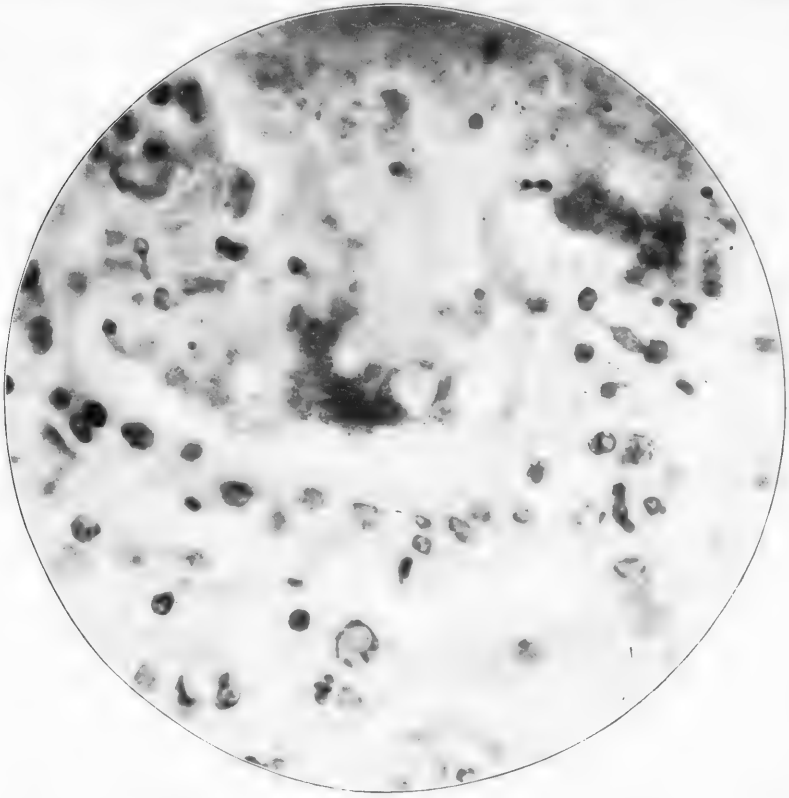

FIG. 1.

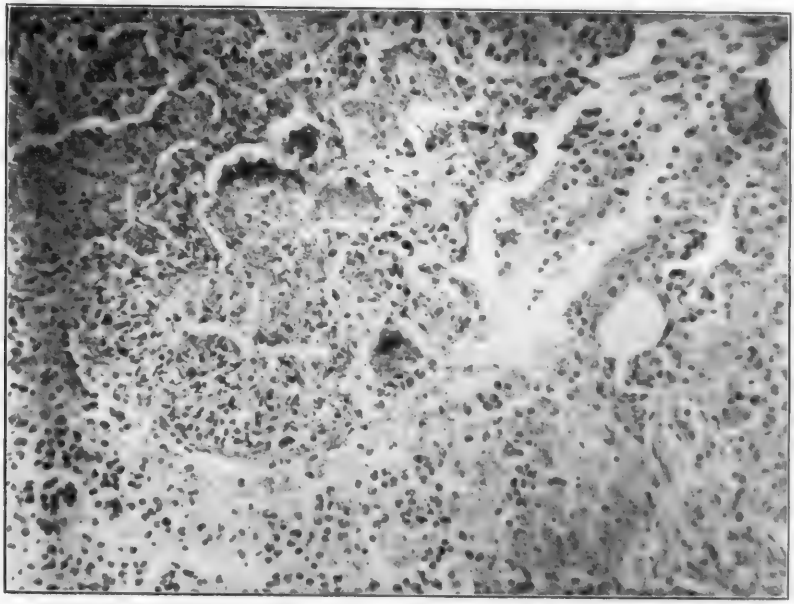

FIG. 2.

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THE EFFECT OF CONDITIONS UPON THE LATENT PERIOD AND RATE OF ASEPTIC POST-MORTEM AUTOLYSIS DURING THE FIRST TEN HOURS.[1]

By HOLMES C. JACKSON.

Adjunct Professor of Physiological Chemistry, Albany Medical College, Albany, N. Y.

The experiments about to be described had for their purpose an attempt to determine the character of the initial physical or chemical changes which occur in an organ as the result of its becoming disconnected from the blood supply, and are responsible for the setting up anew or the gaining ascendency of those catalytic processes commonly known as autolysis. It seemed that information gained in this direction would be of especial value in a discussion of the question as to whether autolytic processes present but a survival of the same or similar processes occurring in the living cell or whether they represent reactions set up *de novo* as the result of incipient somatic death.

The point of attack in such a problem must fall upon the initial or early hours of the autolytic process. On this acount, one may neglect in the discussion the almost numberless investigations dealing with autolytic processes in general and the effect upon them of changes in condition such as reaction, etc., and confine oneself to the experiments in which the autolysis was studied with especial reference to the first six to eight hours after shutting off the circulation from the organ either by removal from the body or otherwise.

But few investigators have busied themselves directly with this line of work. Of first importance must be considered the work

[1] Conducted under a grant from the Rockefeller Institute for Medical Research. Read by title before the American Society of Biological Chemists, Chicago, Ill., January 2, 1908. Abstract appeared in the *Journal of Biological Chemistry*, 1908, iv, p. xxxvii. Received for publication October 12, 1908.

of Lane-Claypon and Schryver (1) which indicates that the course of autolysis in the first twenty-four hours may be divided into three stages; (*a*) a latent period of from two to four hours, (*b*) a period of rapid autolysis and (*c*) a period of gradual autolysis.

Of perhaps chief interest in this connection is the latent period of two to four hours existing between the removal of the tissue from the body and the commencement of autolysis. If such a latency in autolysis does exist and is not the result of some condition of the experiment, then it becomes natural to conclude that this latent period represents the time during which the condition of the cell is being so changed by the lack of circulating blood and perhaps other causes that new or more rapid catalytic reactions may take place and set up a disintegration more pronounced than occurs during cellular life. Such reactions may be originated in various ways. Lane-Claypon and Schryver found that the state of nutrition markedly influenced the course of the autolysis in that the latent period was absent in the livers of fasting animals and that these tissues underwent more rapid decomposition than was the case in the well-fed animal. This is explained with the assumption that since it is probable that enzymes are liberated in the process of autolysis, the fasting liver contains more enzymes free to act (uncombined) or in the form of corresponding zymogen. The latent period is taken as indicating that in the well-fed animal a preponderance of the enzymes are in the form of zymogens and that the first stage in autolysis consists in the transformation of zymogen into active zymin. The presence of free enzymes in fasting animals indicates to these authors the probability that the liberation of these enzymes acts as a normal mechanism for the *intravitam* utilization of proteins in tissues—the supplying of food when none is taken in per os.

In a subsequent paper Schryver (2) has somewhat altered his theory of enzyme liberation as a cause of the latent period in order to correspond to criticisms of Wiener (3), who claimed that the latent period was merely due to the natural inhibitory effect of the alkaline reaction of the living tissues and that the latency ended with the sufficient neutralization of the alkalies by means of the acids formed in autolysis. This, of course, is reasoning in a circle

since it does not explain what originates or accelerates the process by which the acids are formed for neutralization purposes.

Schryver has advanced a somewhat complicated theory for the preservation of chemical equilibrium in the living cell. The equilibrium of enzymes is established by the presence of an excess of ammonia which prohibits the formation of an acid reaction, necessary for autolysis. During starvation, the excess of ammonia gradually disappears and when the production of acid is sufficient to supersaturate the alkalinity, the autolytic enzymes liberate amino-acids; these in turn are transformed in the liver with the production of ammonia which again inhibits the autolytic processes. The non-nitrogenous moiety of the autolytic products serves as food for the fasting cells.

It can hardly be conceived that the author believes that free ammonia is present in the cell at any time, although this is necessary to the theory. In view of the neutrality of protoplasm and the extreme variations which may occur in the production of acid and of alkali without change in this neutrality as shown by Henderson and Black (4), Schryver's position seems untenable.

On account of the many possible explanations for the latent period and its importance in deciding the cause of autolysis, it seemed of sufficient interest to examine this subject somewhat more closely and to attempt if possible to shorten or prolong the period by altering the conditions of the experiment.

In order to obtain an insight into the character and velocity of the reactions taking place in the liver after its removal from the body, three factors were determined during the autolyses.

1. The changes in the depression of the freezing point. This method has been employed by several authors and more recently by Fredericq (5), Delrez (6) and by Wells and Benson (7). The first of these investigators employed a somewhat complicated procedure in the preparation of the tissue for examination. The methods seem faulty and have been criticized by Wells. The latter two authors have determined coincidently the changes in the electrical conductivity and depression of the freezing point during autolysis. Both authors report that in general these factors parallel each other closely. In this work, therefore, the determination of the electrical conductivity has for various reasons been neglected.

2. The increase or decrease in non-coagulable nitrogen.

3. Changes in reaction were examined by means of phenolphthalein and dimethylaminoazobenzene. Body fluids react acid to the former and alkaline to the latter; phenolphthalein is particularly sensitive to changes in the hydrogen ionic content while dimethylaminoazobenzene only indicates an H^+ concentration about equal to that of an 0.8 per cent. lactic acid solution. In this way it seemed possible to determine the character of the substances causing the change in reaction, if such occurred.

The general method of experimentation was as follows: the liver tissue was removed aseptically,[2] passed through a sterile hashing machine into a sterile Erlenmeyer flask of 500 cubic centimeters capacity provided with a cotton plug. The flask containing 300 cubic centimeters of the salt solution (cold or at 38° C.) in which the autolysis was to proceed was counterpoised on scales and 100 grams of the ground tissue were weighed out by allowing the material to drop from the machine directly into the flask. During the process the whole apparatus was covered with a sterile cloth to exclude air contamination. The mixture being thoroughly shaken a control sample of 50 cubic centimeters was removed with bacteriological technique into a smaller stoppered flask. This was brought to the boiling point without change in reaction, allowed to cool, thoroughly mixed, and filtered.

This coagulum-filtrate was immediately put through the routine examination for determination of the depression of the freezing point by means of Beckmann's apparatus,[3] determination of the non-coagulable nitrogen by the Kjeldahl method and titration of the respective acidity by means of phenolphthalein and alkalinity to dimethylaminoazobenzene. For each of these determinations ten cubic centimeters of the filtrate were employed and all were conducted in duplicate.

After the removal of the control sample the flask was placed in the thermostat at 38° C; when the liver was not irrigated this

[2] The usual surgical operative technique was employed.

[3] Several control examinations were made as to the depression of the freezing point of the coagulated and uncoagulated samples. The two gave results which were almost identical or within the limit of accuracy of the work.

occurred about twenty minutes after the death of the animal; the washing of the organ occupied ten minutes longer time. At definite times as indicated by the protocols, samples of 50 cubic centimeters were removed with bacteriological technique, the sample coagulated as above and the main flask returned to the incubator. At each time when a sample was withdrawn, a drop of the autolytic mixture was allowed to fall upon a sterile agar slant and at the end of the experiment these were incubated at 38° C. for from twenty-four to forty-eight hours. In practically all cases where the experiments ran for only six to eight hours, the agar slants when examined by a stained smear gave negative results—indicating a practical absence of organisms or, at least, that they were in such small numbers that a drop of the mixture taken at random failed to include one of them. All experiments in which the slant or smear indicated organisms, have been excluded. In the experiments where the period was extended, it was noticed that at times at the twelfth hour there was evidence of the presence in small numbers of certain organisms. In some instances the tests would remain negative even at twenty-four hours; in others the hay bacillus or another bacillus which will be discussed in a short note by Jackson and Hawn, made its appearance between twelve and twenty-four hours in quantities sufficient to give a decidedly sour non-putrefactive odor to the autolytic mixture. In this connection must be mentioned the work of Magnus-Levy (8) who showed that acids of fatty nature as well as methane and hydrogen sulphide are produced during the first twenty-four hours of aseptic liver autolysis. His results as regards acidity are confirmed in the work presented here. This author also experienced the same difficulty in obtaining sterile organs that was met with in this work. Experiments in which infection has occurred have either been omitted or a note of the infection made in the protocol. In some other experiments a decided putrefactive odor appearing between six and eight hours indicated contamination of some sort; no account has been taken of these.

Note must also be made of the fact mentioned by Schryver (9) and others that very frequently the glycogen when present in the liver of the dog disappeared very slowly during the autolysis. In

certain experiments it was still present at the end of six hours of incubation; at times, however it would disappear earlier, but no instance can be found in the notes where the first or third hour sample was negative when the control indicated the presence of glycogen. Schryver states that in fasting dogs the glycogen disappears very quickly, while it remains for twenty-four hours in the livers of well-fed animals. It is hard to explain why livers of animals which have fasted for several days should contain glycogen at all, since it is ordinarily believed that in starvation it disappears quite quickly. In only some of the fasting animals in the experiments of this paper did glycogen appear in the liver; it is therefore manifestly impossible to make comparison with Schryver's results. The variations in appearance of glycogen and the rapidity of its disappearance noticed in the well-fed animals renders Schryver's distinction improbable however. The probable relationship of the slow disappearance of glycogen from the liver post-mortem and of its presence or absence to the autolytic processes going on, is discussed elsewhere in this paper.

Again in certain autolyses the mixture after the second or fourth hours began to take on a greenish nuance which developed quite rapidly in intensity until at the sixth hour, the color became dark green, almost black. This was noticed, of course, in a comparison of the coagulum-filtrates at the end of the experiments and seemed most prone to occur in those cases where the liver contained glycogen; the phenomenon remained present throughout the autolysis. Without doubt this peculiar condition was the result of the presence of oxidizing enzymes similar to tyrosinase and to those which, noticed in plants by Palladin (10) and others, transform colorless into dark colored compounds, pigments or melanines.

As evidence of the accuracy of the experiments and the limits of error which the technique involved, reference must be made to the protocols of two experiments No. 25A, B and C and 26A, B and C.[4] In these cases three controls were simultaneously started the only difference being that two of them in each experiment were kept in the dark. Since this condition did not seem to effect the autolyses, the results serve as admirable controls of the method.

[4] See Tables II, III and IV.

The experimental evidence obtained as the result of this work will be taken up as follows:

1. Effect on latent period of (*a*) condition of animal, (*b*) presence or absence of blood, (*c*) temperature of diluent salt solution.

2. Effect of conditions upon the commencement and rate of the autolysis. (*a*) nutritional state of the cell, (*b*) light, (*c*) antiseptics, (*d*) reaction, (*e*) salt concentration.

LATENT PERIOD.

Effect of Nutrition.—Schryver's experiments indicated that the best conditions for obtaining a latent period were present in the livers of well-fed animals. Hence Series I (four experiments) was carried out after the general method indicated previously in the paper. The animals were well-fed for several days previous to death; the livers were taken out without irrigation and *cold* physiological salt solution (Δ 0.568) added in appropriate amount. The whole mixture of tissue and salt solution was then placed in the thermostat at 38° C. All experiments (5a, 5b, 6, 10) showed (Table I) that the depression of the freezing point remained practically the same as the control with a slight tendency perhaps to decrease until the third or the fourth hour after which a more or less marked increase occurred to the end of the experiment. The figures for the non-coagulable nitrogen although somewhat fragmentary indicate also a positive decrease from the control occurring during the first three or four hours. The figures for the fifth and sixth hours rising above the control are indicative of the commencement of nitrogenous autolysis. The acidity to phenolphthalein increased without much evidence of latency except in Experiment 6 while the figures for the alkalinity to dimethylaminoazobenzene vary—two experiments showing no change, one an increase and one a decrease. The figures considered as a whole would certainly convey the impression that autolytic changes of the usual type had not begun to show themselves until between the third and fourth hour after removal of the tissue from the animal's circulation.

In the endeavor to control the statement of Schryver that the fasting livers did *not* show this latent period, Series II of three

TABLE I.[5]

Series I.

Liver Unwashed; Cold Saline Solution.	Control.	1 Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.	6 Hours.	7 Hours.
Exp. 5a	0.670	0.655	0.665	0.680	0.690	0.715	0.750	
	6.20	5.20	5.25	5.05	5.35	5.95	6.60	
Well-fed	0.55	0.60	0.65	0.70	0.75	0.85	1.85	
	0.85	1.00	1.15	1.30	1.45	1.65	2.20	
Exp. 5b	0.670	—	0.665	0.665	0.685	0.685	0.695	0.710
	—	—	5.65	5.75	5.55	5.55	5.65	5.85
Well-fed	0.30	0.40	0.40	0.35	0.55	0.60	0.65	0.70
	1.00	1.05	1.00	0.95	1.00	1.00	0.95	0.95
Exp. 6	0.665	0.650	0.660	0.655	0.665	0.665	0.675	0.690
	7.00	6.15	5.90	6.60	7.80	8.00	8.30	—
Well-fed	0.50	0.50	0.50	0.60	0.65	0.80	0.85	0.90
	1.20	1.10	1.15	0.95	0.90	0.95	0.95	1.00
Exp. 10	0.675	—	0.660	0.660	0.675	0.685	0.720	0.725
	7.45		6.85	7.35	7.60	7.85	7.60	7.70
Well-fed	0.75	—	0.90	1.05	1.05	1.10	1.30	1.35
	1.35	—	1.40	1.25	1.35	1.30	1.55	1.40

Series II.

Exp. 14	0.600	0.595	0.605	0.615	0.615	0.620	0.645
	7.35	7.45	7.70	7.75	7.85	7.90	8.45
Not fed	0.75	0.90	1.05	1.05	1.10	1.30	1.35
	1.35	1.40	1.25	1.35	1.30	1.55	1.40
Exp. 22	0.660	0.685	0.710	0.700	—	0.710	0.715
	6.95	7.20	7.10	6.90	7.20	7.20	7.10
Not fed	0.40	0.50	0.70	0.70	0.95	0.90	1.10
	0.90	0.95	1.00	0.90	1.05	1.10	1.00
Exp.28A	0.235	0.235	0.240	0.290	0.330	0.340	0.345
	5.80	4.65	4.55	4.35	4.45	44.0	4.55
Not fed	0.40	0.55	0.45	0.60	0.85	1.05	1.35
	1.35	1.15	1.25	1.20	1.25	1.10	1.35
Exp. 19	0.670	0.710	0.720	0.725	0.735	0.755	0.790
	5.95	6.00	5.75	5.75	6.00	6.30	7.50
Well-fed	not done						

experiments (Nos. 14, 22, 28A) were conducted in a manner similar in all details to Series I except that the animals were fasted for a few days previous to use. The livers were free from glycogen with the exception of that used in Experiment 28A.

[5] The upper left-hand figures represent degrees of depression of the freezing-point; the upper-right-hand figures, non-coagulable nitrogen expressed as cubic centimeters of *N*/10 acid; the lower left-hand figures, acidity to phenolphthalein in terms of cubic centimeters of *N*/10 acid; lower right-hand figures, alkalinity to dimethylaminoazobenzene in terms of cubic centimeters of *N*/10 alkali.

In the protocol (Table I) the results for the depression of the freezing-point show that in two experiments a latent period of two hours existed while in the third (Experiment 22) autolysis began immediately. The figures for non-coagulable nitrogen in Experiments 22 and 28A, however, point strongly to an inhibition of autolysis which lasted for five hours. In Experiment 28A the animal had not received food for three days previously; the liver contained glycogen which, however, gradually disappeared until no evidence of it could be obtained at the fourth hour, at which time it will be noticed that the marked depression of the freezing-point stopped. The non-coagulable nitrogen of the successive samples showed a marked diminution over the control with no evidence of an increase—rather a decrease—to the end of the experiment. This peculiar relation of glycogen disappearance to changes in the depression of the freezing-point and non-coagulable nitrogen will be taken up later in the paper. Finally one experiment (No. 19) with a well-fed animal similar to those of Series I gave no latent period as indicated by the depression of the freezing-point but the non-coagulable nitrogen figures showed an inhibition of autolysis lasting until the fourth hour. In all of the experiments in Series II the acidity figures with phenolphthalein showed a consistent increase from the very beginning of the experiment, while the alkalinity to dimethylaminoazobenzene remained constant to the end.

Although Series I with well-fed animals resulted as expected according to Schryver, Series II with three fasting animals and one well-fed animal was unsatisfactory.

Turning about to find a cause for these varying results and at the same time attempting to obtain some condition which might influence the latency of the autolysis, it seemed plausible to try the effect of two factors: first, the absence of blood; and second the use of *warm* (38° C.) saline at the outset of the experiment.

Effect of Blood.—The elucidation of the question as to the effect of blood upon the latent period would bear directly upon the criticism of Schryver by Wiener mentioned above, as well as serve to accentuate the fact of the well-known inhibitory effect of blood upon the rapidity of autolysis noticed so frequently[6] although its

[6] Schryver (p. 180), in one experiment, found no difference in the rate of autolysis between the perfused organ and one containing blood.

cause is so poorly understood (11). The washing of the alkaline blood from the liver would tend certainly to remove a certain amount of bases from the organ together with other salts lost by diffusion during irrigation with the salt solution. Consequently two experiments (Series III) were conducted in which the livers were thoroughly irrigated through the portal vein with cold saline solution and diluted with cold saline solution; one animal was fasting (No. 20) and the other well-fed (No. 17). From the figures (Table II) for the depression of the freezing-point no latency of autolysis occurred and the increase is pronounced to the end of the experiment. The same is true for the non-coagulable nitrogen except that a slight indication of possible latency showed itself in the well-fed animal. The acidity to phenolphthalein increased and the alkalinity to dimethylaminoazobenzene remained constant as usual.

These two preliminary experiments seemed sufficient to make it evident that the presence of blood formed one of the inhibitory factors in the latency. This fact is of course not new except as it applies to the latent period and was to be expected since, as was stated above, the inhibitory effect of alkalies upon autolysis and their supposed action in prohibiting autolysis *in vivo* might be considered as persisting some time after removal of the organ after death.

Effect of Temperature of Salt Diluent.—The next series (IV) was planned to determine the effect of keeping the organ at body temperature during removal and subsequent autolysis. The washing was therefore accomplished with *warm* saline and *warm* saline added as diluent. The only time during which a lowering of temperature could occur was during the period of hashing. This, of course, represented only a few minutes and could cause at most a temperature depression of a few degrees. Some of the hashed material was tested and gave 34.5° C. The warm saline quickly brought this up to 37° C. Seven experiments were carried on in this way (Experiment 9, 11, 15, 18, 29E, 26A, 26C). Of these the first four were well-fed and the last three fasting animals. Of the well-fed animals two (9, 18) showed (Table II) a marked autolysis at the outset as indicated by the figures for the depression of the freezing-point and nitrogen. On the other hand in

TABLE II.[1]

Series III.

Liver Washed; Cold Saline.	Control.	1 Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.	6 Hours.
Exp. 20	0.555	0.570	0.590	0.595	0.600	0.610	0.625
	5.40	5.90	5.80	6.10	6.30	6.40	6.60
Not fed	0.35	0.40	0.70	0.75	0.80	0.95	1.05
	0.70	0.65	0.70	0.80	0.85	0.80	0.90
Exp. 17	0.565	0.585	0.600	0.620	0.640	0.650	0.710
	5.05	4.95	5.15	5.50	5.65	5.80	6.30
Well-fed	0.50	0.60	0.70	0.80	0.85	1.00	1.10
	1.00	0.90	0.95	0.95	1.20	1.15	1.05

Series IV.

Liver Washed and Diluted with Warm Saline.	Control.	1 Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.	6 Hours.
Exp. 9	0.575	0.595	0.605	0.620	0.635	0.665	0.670
	5.70	6.35	7.05	7.35	8.65	9.65	10.50
Well-fed	0.50	0.85	1.15	1.15	1.25	1.35	1.60
	1.15	1.20	1.40	1.50	1.50	1.50	1.95
Exp. 11	0.555	0.540	0.585	0.590	0.590	0.615	0.640
	5.40	5.35	5.50	5.70	6.15	6.30	6.80
Well-fed	0.50	0.65	0.80	0.80	0.90	1.00	1.10
	1.10	1.25	1.05	0.95	1.00	1.25	1.35
Exp. 15	0.615	0.605	0.615	0.635	0.640	0.645	0.670
	6.30	6.10	6.20	6.35	6.50	7.20	8.25
Well-fed	0.40	0.55	0.60	0.75	0.90	1.00	1.10
	1.00	1.00	0.95	0.95	1.00	1.25	1.35
Exp. 18	0.660	0.675	0.705	0.710	0.725	0.735	0.740
	5.30	5.85	5.90	6.30	6.70	6.80	7.25
Well-fed	0.40	0.60	0.65	0.70	0.85	0.90	1.05
	0.60	0.95	0.95	0.75	0.75	0.65	0.75
Exp. 29E	0.325	0.345	0.365	0.385	0.395	0.415	0.435
	4.50	--	5.70	—	6.45	—	7.45
Not fed	0.45	0.65	0.80	0.95	1.10	1.20	1.40
	0.75	0.80	0.80	0.70	0.75	0.75	0.70
Exp. 26A	0.560	0.565	0.575	—	0.585	0.600	0.625
	4.75	5.10	5.55	6.00	6.50	7.15	7.65
Not fed Dark	0.35	0.50	0.75	0.80	0.85	0.90	1.00
	0.60	0.70	0.65	0.60	0.70	0.75	0.80
Exp. 26C	0.555	0.565	0.570	0.585	0.610	0.620	0.625
	4.90	4.95	5.25	6.30	6.75	7.00	7.45
Not fed	0.20	0.35	0.55	0.75	0.90	0.95	1.05
	0.50	0.75	0.85	0.80	0.75	0.80	0.85

[1] The upper left-hand figures represent degrees of depression of the freezing-point; the upper right-hand figures, non-coagulable nitrogen expressed as cubic centimeters of *N*/10 acid; the lower left-hand figures, acidity to phenolphthalein in terms of cubic centimeters of *N*/10 acid; lower left-hand figures, alkalinity to dimethylaminoazobenzene in terms of cubic centimeters of *N*/10 alkali.

Experiments 11 and 15 a well-marked latent period was present although it persisted for only one hour. Only traces of glycogen were present in the liver of Experiment 11 and it was absent in Experiment 15. The fasting animals whose livers were irrigated and diluted with warm saline gave figures both for depression of the freezing point and nitrogen indicating no latency and an autolysis which was the most marked of all the experiments of this type.

A fifth series of four experiments (25A, 25C, 31C and 36A) were conducted with unwashed livers of well-fed animals employing warm saline as diluent. Three showed (Table III) immediate

TABLE III.[8]

Series V.

Unwashed Liver, Well-fed, Warm Saline.	Control.	1 Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.	6 Hours.
	0.730	0.745	0.775	0.790	0.810	—	0.840
Exp. 25A	5.10	5.40	5.60	5.70	5.80	—	6.30
	0.30	0.50	0.60	0.65	0.75	—	0.95
Dark	0.65	0.55	0.65	0.80	0.90	—	1.20
	0.725	0.755	0.780	0.795	0.810	0.825	0.835
Exp. 25C	4.65	4.90	4.95	5.10	5.20	5.65	5.85
	0.40	—	0.55	0.65	0.70	0.85	0.95
	0.50	0.60	0.70	0.85	0.90	1.05	1.10
	0.530	0.590	0.605	0.625	0.645	0.660	—
Exp. 31C	6.10	6.20	6.25	6.35	6.70	7.00	—
	0.50	0.60	0.70	0.75	0.85	0.85	0.95
	0.70	0.80	0.80	0.65	0.80	0.60	1.35
	0.720	0.715	0.735	0.755	0.775	0.785	0.800
Exp. 36A	6.05	5.60	5.50	5.90	6.15	6.65	7.15
	0.40	0.70	0.75	0.80	0.85	0.85	0.85
	1.50	1.30	1.15	1.25	1.20	1.10	1.20

autolysis both in the figures for depression of the freezing-point as well as for non-coagulable nitrogen; one (36A) did not autolyse during the first three hours according to the nitrogen figures, the depression of the freezing-point only indicating an inhibition for

[8] The upper left-hand figures represent degrees of depression of the freezing-point; the upper right-hand figures, non-coagulable nitrogen expressed as cubic centimeters of $N/10$ acid; the lower left-hand figures, acidity to phenolphthalein in terms of cubic centimeters of $N/10$ acid; lower right-hand figures, alkalinity to dimethylaminoazobenzene in terms of cubic centimeters of $N/10$ alkali.

the first hour only. It is questionable whether an autolytic latency existed here.

The following classification of experiments in the five series will indicate the results at a glance.

Conditions of Experiments.	Latent Period. Positive.	Negative.	
Unwashed, well-fed, cold saline.....	4	0	Series I
Unwashed, fasted, cold saline.....	2	0	Series II
Unwashed, well-fed, cold saline.....	1?	1	Series II
Washed, fed, cold saline.....	0	1	Series III
Washed, fasted		1	Series III
Washed, fed, warm saline.....	1	3	Series IV
Washed, fasted, warm saline.....	0	3	Series IV
Unwashed, fed, warm saline.....	1?	3	Series V
	7+2?	12	

Positive Latent Period.

Fed	5+2?	Fasting	2
Cold saline	6+1?	Warm saline	1+1?
Unwashed	6+2?	Washed	1

From the tabulation it will be seen that out of twenty-one experiments nine showed a latent period; in two of these, however, the latency lasted only one hour so that these have been given a question mark. Of the nine positive experiments seven occurred with well-fed animals and only two in fasting ones. These results are somewhat in agreement with Schryver. Again seven of the positive latent periods occurred in experiments where cold saline was employed as diluent. Schryver employed small amounts (4 grams) of material for his experiments. This would cause a rapid lowering of temperature with consequent inhibition. Whether this author used a cold or warm diluent is not stated, and the organs were not washed.

Perhaps some importance may be attached to the fact that five of the nine positive results occurred as the first five experiments performed when the time between the removal of the organ and commencement of the autolysis was somewhat longer than in the latter experiments when our general technique had improved. In this connection it is proper to state that of twenty-five other and similar experiments reported below only four showed a latent period before autolysis and of these, cold diluents were employed in three cases.

In view of these facts and of the fact that of thirteen latent periods, nine were in experiments where cold diluents were used, it is evident that the low temperature of the mixture acts as an inhibitory factor in causing a latent period of autolysis until the material becomes warmed throughout to body heat. The presence of blood in the unwashed organ adds an additional factor to the inhibiting forces.

Upon the whole, therefore, it seems that the latent period is most prone to appear in unwashed livers where cold diluent is employed and in those cases in which the animal was previously well fed. It seems, however, that in the light of all the experiments herein presented, it becomes questionable whether the feeding of the animal and the consequent condition of the liver possesses any direct influence on the latency of autolysis.[9] In this it is impossible to agree with Schryver nor does the latter's reasoning in regard to the liberation of enzymes in the fasting condition seem plausible. There does not exist at present, any evidence which points to any qualitative difference in the enzymic condition between the metabolism of well-fed and fasting animals. The statement that in fasting, autolytic processes by causing decomposition supply the needs of the body by the utilization of the degenerative products simply amounts to placing a well established fact in new terms, since it is very difficult to draw a sharp line between the normal analytic processes by which energy is liberated and those of autolysis associated with the death of the cell. It would appear that the question was more one of a disturbed quantitative equilibrium of the cell than of any qualitative enzymic differences.

EFFECT OF CONDITIONS UPON THE RATE OF AUTOLYSIS.

The previous discussion has had to do with autolysis within the first three or four hours after the removal of the organ from its circulation. The fact that the results indicated that the latent period was relatively inconstant and dependent upon a variety of factors led to the attempt to study first, the rate of autolysis within the first twelve hours and second, the effect upon it of altering cer-

[9] As explained later in the paper, the nutritional state of the cell may indirectly cause autolytic variations.

tain conditions in the experiment in the hope that the onset of changes favoring autolysis might be inhibited. This consisted mainly in endeavoring to continue the alkalinity of the cell by means of alkaline phosphates.

Nutritional State of the Cell.—One of the most important factors to be considered in the elucidation of the origin and rate of autolysis consists in the determination of the level of equilibrium upon which the cell exists at the time of stoppage of the circulation. We look to the diet of the animal as one of the most ready means of normally altering this condition and yet it becomes one of the most difficult to control, since no two animals react similarly to diet or to fasting.

The fat content of the livers of normal animals under the same diet varies as greatly as does the power of individuals to lay on subcutaneous fat.

The appearance or absence of glycogen serves perhaps as best evidence concerning the condition of the cell from the standpoint of nutrition and it is most usual to conclude that the tissues are actually in a state of undernutrition when the liver is free from glycogen. Well-fed animals usually possess organs containing more or less glycogen, but even here exceptions exist and frequently animals after a long period of excessive carbohydrate diet do not show glycogen in their livers.

Concerning variations in the nitrogenous content of the liver, it becomes more difficult to make definite statements. Pearce and Jackson (12) showed that as the result of incipient necrosis or starvation from lack of circulation the cells involved lay on nitrogenous compounds in the same way as they do fat. This was in agreement with the results of Seitz (13) obtained under normal conditions. Schryver states that the liver of a fasting dog contains more non-coagulable nitrogen than that of a well-fed animal. In the experiments here reported a great variation in the amount of initial non-coagulable nitrogen exists. The average figure for twenty experiments upon fasting animals compares within one per cent. of that for eighteen well-fed animals. Selecting two cases (Experiments 25 and 26) in each of which there are three controls, it is evident that no difference exists. It is not possible, therefore,

to confirm Schryver's statement in regard to differences in nitrogen.

From a consideration of the initial figures for the depression of the freezing-point in twenty-nine experiments in which physiological saline was employed as diluent it is evident that the fasting liver possessed the lowest concentration. Eleven washed organs of which six were fasting and five well-fed and eighteen unwashed organs, seven fasting and eleven well-fed gave averages as follows:

Washed, fasting 0.475° (310–560°)	Unwashed, fasting 0.609° (575–660°)
Washed, well-fed 0.594° (555–660°)	Unwashed, well-fed 0.664° (515–735°)

These figures seem sufficiently definite to indicate that as far as ionic concentration is concerned the fasting animals'[10] livers showed the lowest figures whether washed or unwashed. The maximum and minimum effects are indicated in Experiments 25 and 26. Experiment 25 was an unwashed well-fed liver and gave, $\Delta =$ 0.730 (average three controls) while Experiment 26 with washed and fasting organs, $\Delta = 0.555$ (average three controls). In all instances the washing of the organ decreased the ionic strength of the tissue; this procedure seemed to have little effect however upon the initial acidity or alkalinity. From the fact that perfusion of an organ causes a decreased ionic concentration which is not paralleled by changes in reaction, it seems probable that the ionized substances in the cell which impart its so-called alkaline reaction are of such a character (ionized protein) that interchange or osmosis through the endothelium does not readily take place.

There are no data obtainable from the results presented in this paper which can be of service in explaining the effect of these variable nutritional conditions of the cell upon the latent period and rate of autolysis. The cursory outline above serves merely to bring to mind possible variations in cell equilibrium as regards the carbohydrates, fats, proteins, osmotic concentration, etc. This phase of the subject will receive further consideration in the final discussion as to the cause of and variations in the changes in the reaction of the cell occurring at its death.

Light.—The first external change in condition which usually

[10] Animals were given water *ad libitum.*

occurs in tissues undergoing incipient autolysis *in vitro* and which is not in accordance with autolysis *in vivo* consists in the placing of the material in the light. Two experiments (25 and 26)—one a well-fed and the other a fasting animal—were consequently performed in which the liver was removed in the dark and two of the samples (25A and B and 26A and B)[11] autolysed in the

TABLE IV.[11a]

Autolysis in the Dark; Cold Saline (Control).	Control.	1 Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.	6 Hours.
Exp. 25B	0.735	0.755	0.765	0.780	0.810	0.820	0.835
	4.80	5.50	5.65	5.85	5.95	6.00	6.10
Not-washed; well-fed	0.40	0.50	0.65	0.60	0.65	0.70	0.75
	0.65	0.70	0.85	0.80	0.85	0.80	0.85
Exp. 26B	0.550	0.550	0.560	0.575	0.590	0.615	0.630
	5.20	5.35	5.85	6.10	6.60	7.15	8.00
Washed; not fed	0.35	0.30	0.50	0.60	0.70	0.95	1.00
	0.55	0.65	0.80	0.70	0.65	0.85	1.05

Washed; Not-fed.	Control.	2 Hours.	4 Hours.	6 Hours.	8 Hours.	10 Hours.
Exp. 29E	0.325	0.365	0.395	0.435	0.485	0.565
	4.50	5.70	5.75	7.45	10.10	15.50
Warm saline	0.45	0.80	1.10	1.40	2.50	2.95
	0.75	0.80	0.75	0.90	0.95	1.95
Exp. 29F	0.310	0.330	—	0.385	0.390	0.395
	4.80	5.90	—	6.45	7.40	8.00
Warm saline and toluol	0.45	0.85	0.80	0.85	0.90	1.05
	0.70	0.85	0.85	0.90	0.85	0.90

Unwashed; Well-fed.	Control.	1 Hour.	3 Hours.	5 Hours.	7 Hours.	12 Hours.
Exp. 31C	0.530	0.590	0.625	0.660	—	1.020
	6.10	6.20	6.35	7.00	7.60	10.70
Warm saline	0.50	0.60	0.75	0.85	0.90	1.45
	0.70	0.80	0.65	0.60	0.80	0.95
Exp. 31B	0.515	0.530	0.600	0.620	0.635	0.690
	5.35	5.80	6.25	6.70	7.00	8.50
Warm saline and chloroform	0.50	0.60	0.70	—	0.95	1.95
	0.90	0.85	0.80	—	1.35	3.35

[11] Experiments 25A and 26A differed from 25B and 26B in that in the former a warm diluent was employed. Experiments 26A and 26C appear as the last two upon Table II; Experiments 25A and 25C, as the first two in Table III.

[11a] The upper left-hand figures represent degrees of depression of the freezing-point; the upper right-hand figures, non-coagulable nitrogen expressed as cubic centimeters of *N*/10 acid; the lower left-hand figures, acidity to phenolphthalein in terms of cubic centimeters of *N*/10 acid; lower right-hand figures, alkalinity to dimethylaminoazobenzene in terms of cubic centimeters of *N*/10 alkali.

absence of light. The third sample (25C and 26C) as control, underwent autolysis as usual. These experiments continued for seven and one-half hours. The results (Table IV) indicate that the presence of ordinary light exerts no appreciable influence upon autolysis. The increase of the depression of the freezing-point, increase of non-coagulable nitrogen and change in reaction ran parallel in both experiments. The differences which one experiment showed as against the other as the result of the feeding of the animal, will be considered later.

Antiseptics.—Autolytic experiments as usually conducted in order to insure complete asepsis entail the employment of antiseptics such as toluol, chloroform, etc. The results of Lang (14), while not very complete, indicate that germicidal agents are not without effect upon the activity of the enzymes in autolysis. Wells and Benson (7) however incline to the view that with certain precautions, chloroform may be employed without inhibitory effect. It is evident that where comparative results are to be obtained, this variable factor should be eliminated. In this work antiseptics were not employed except in Experiments 29 and 31. In the former toluol was utilized and in the latter, chloroform. A glance at the figures in the tables will be sufficient to indicate the inhibitory action of the antiseptic, apparently more potent in the case of toluol than of chloroform. This is evident both in the figures for the depression of the freezing-point and for the non-coagulable nitrogen. Experiment 29 ran for ten hours during which time the depression of the freezing-point increased in the mixture without toluol from 0.325° to 0.565°; while in the toluolized autolysis it increased from 0.310° to 0.385°. In Experiment 31 the similar figures for twelve hours duration are with chloroform 0.515° to 0.690°; without chloroform 0.530° to 1.020°. The nitrogen increase paralleled them closely.

Reaction.—It has been quite definitely decided as the result of the work of many investigators (15) that acids accelerate and alkalies inhibit the rate of autolysis. In the majority of the experiments conducted by these workers a concentration of H^+ or OH^- ions has been employed which is out of all accord with that possible in the cell of the animal body. The blood and protoplasm

are from the standpoint of physical chemistry considered as neutral (4); very slight variations from this condition may cause profound alterations in the general metabolic processes. It seemed, therefore, of more value from a comparative standpoint to attempt to vary the so-called acidity and alkalinity relations of the cells in ways which more closely simulate the normal.

The immense importance which the phosphates play as regulating mediums for reaction in the body has been of late repeatedly emphasized. Mixtures of the monohydrogen and dihydrogen salts may be appropriately made so that the resultant solution is either neutral, alkaline or acid.[12] Hence disodium monohydrogen phosphate was employed as diluent in the hope of increasing the "alkalinity" of the tissue while the dihydrogen monosodium phosphate promised to augment the "acidity." A further method employed for the latter purpose consisted in the addition of pure neutral ethyl butyrate to the autolytic mixture. As autolysis proceeded the lipolysis of the butyrate set free butyric acid, thereby increasing the acidity markedly. This reaction may also be supposed to take place in the living cell but ordinarily sufficient bases are at hand for neutralization. In autolysis (lipolytic), should the bases be lacking, the H^+ concentration would be increased.

An examination of the figures obtained for the control samples as indicative of the character of the initial state of reaction of the tissue indicates that either the method of titration with the two indicators is too crude to show slight variations which might occur in the living tissue, or the factors which influence the reaction of the tissue and which would aid in explaining the results, are unknown. Certain differences in the figures for the controls under the various conditions did appear but no one condition which has been discussed here is apparently accountable for the changes.

In Experiments 23, 28B, 30A, 32C, 33B and 36 (Table V) attempts were next made to increase the alkalinity of the reaction of the mixture in which autolysis was to occur by means of disodium hydrogen phosphate in five per cent. solution and Experiment 28C

[12] In relation to phenolphthalein. Henderson (4) has shown that in solutions of these two salts great variations in their relative proportions may exist without changing the H^+ concentration to any great degree.

TABLE V.[13]

5 Per Cent. Na_2HPO_4.	Control.	1 Hour.	3 Hours.	6 Hours.	8 Hours.	10 Hours.
Exp. 23	0.525	0.520	0.560	0.610		
Washed; well-fed; cold	—	5.30	5.60	5.80		
diluent	1.45	1.90	2.00	2.25		
	11.50	11.35	11.30	11.80		
Exp. 28B	0.530	0.545	0.615	0.640		
Unwashed; not fed;	4.30	5.60	7.10	7.75		
warm diluent	0.65	1.00	1.40	1.65		
	11.45	11.25	11.40	11.80		
Exp. 30A	0.685	0.710	0.745	0.780	0.795	0.900
Unwashed; not fed;	6.70	6.65	6.50	7.65	—	12.45
10 per cent. warm	0.70	1.00	1.05	1.15	1.70	2.40
diluent	21.75	21.65	20.90	20.95	20.05	19.50
Exp. 32C	0.735	0.705	0.705	0.715		
Unwashed; not fed;	—	—	—	—		
cold diluent	—	—	—	—		
	—	—	—	—		
Exp. 33B	0.700	0.730	0.740	0.790		
Unwashed; not fed;	8.55	8.15	8.20	8.80		
cold diluent	0.90	1.10	1.60	2.50		
	10.3	10.6	10.1	9.65		
Exp. 36	0.910	0.900	0.950	0.995		1.620
Unwashed; well-fed;	6.15	6.40	6.90	7.55		17.55
warm diluent	0.90	1.20	1.55	1.75		17.55
	13.10	14.90	13.05	12.65		26.4
Exp. 28C	0.680	0.700	0.785	0.795	0.805	
Unwashed; not fed;	5.60	4.40	4.65	4.80	4.60	
warm	1.60	1.70	1.85	1.70	1.75	
alkaline Locke's solution	1.15	1.15	—	1.50	1.25	
Exp. 32B	0.650	0.660	0.650	0.650		
Unwashed; not fed;	—	—	—	—		
	—	—	—	—		
Cold Na_3PO_4	—	—	—	—		

by alkaline Locke's solution.[14] It is interesting to note that while the disodium phosphate solution was *alkaline* to phenolphthalein (0.8 c.c. $N/10$ acid for 10 c.c. of solution) and alkaline to dimethylaminoazobenzene (10.65 c.c. $N/10$ acid for 10 c.c. of solution) when the autolytic mixture of this solution and tissue was analyzed, the mixture possessed the *acidity* to phenolphthalein ordinarily

[13] The upper left-hand figures represent degrees of depression of the freezing-point; the upper right-hand figures, non-coagulable nitrogen expressed as cubic centimeters of $N/10$ acid; the lower left-hand figures, acidity to phenolphthalein in terms of cubic centimeters of $N/10$ acid; lower right-hand figures, alkalinity to dimethylaminoazobenzene in terms of cubic centimeters of $N/10$ alkali.

[14] Locke's solution to which was added sufficient sodium bicarbonate to bring the strength of the salt up to 0.5 per cent.

found when sodium chloride is employed as diluent. Unfortunately, it is impossible to say from the methods employed just what physical changes occurred when the tissue and phosphate solution were mixed. From the chemical standpoint of reaction the tissue transformed the alkaline solution (phenolphthalein) to one which reacted acid. The explanation for this probably lies in the rearrangement of phosphates in the solution by which the large excess of disodium hydrogen phosphate reacted with acid factors in the tissues and became transformed into sufficient dihydrogen sodium phosphate to cause the autolytic mixture to assume its normal reaction. This forms simply another manifestation of the regulating mechanism described by Henderson (4). All attempts, therefore, to increase in this manner the alkalinity (phenolphthalein) were negative and the results of these experiments do not differ from those reported with saline as diluent.

One experiment performed with Locke's solution made alkaline with 0.5 per cent. sodium bicarbonate[15] (28C) ran seven and one-half hours. In this instance also the resultant mixture of liver and bicarbonate solution became acid to phenolphthalein and alkaline to dimethylaminoazobenzene. In fact the respective acidity and alkalinity were greater in each case than with sodium chloride. Undoubtedly a reaction occurred between the phosphates of the cell and the sodium bicarbonate, carbon dioxide being liberated, and in this way increasing the acidity to phenolphthalein.

The increase in depression of the freezing-point[16] was about that observed in other experiments, where a similar reaction was present; no inhibition or latent period could be noticed. An examination of the figures for non-coagulable nitrogen indicates, however, that no nitrogenous autolysis occurred, the figures for the end of the experiment being the same as the control or even somewhat lower. In this case no increase in acidity (phenolphthalein) took place such as ordinarily occurs with sodium chloride solution as diluent.

One experiment employing tri-sodium phosphate as diluent did not result favorably since the autolytic mixture became extremely

[15] Of this solution, 10 c.c. required 0.15 c.c. $N/10$ acid to neutralize to phenolphthalein and 1.55 c.c. to dimethylaminoazobenzene.

[16] Whether this is indicative of autolysis is questionable; *c. f.*, the later discussion on this point.

thick and ropy. Upon heating no coagulation occurred; the mixture required the addition of such a large amount of acid before complete coagulation occurred that attempts to perform nitrogen and acidity determinations were abandoned. Unfortunately the diluent solution was employed cold so that it is impossible to decide whether the extremely long "latent" period as evidenced in the depression of the freezing-point was to be ascribed to this or to the phosphate solution. It would seem more probable that the lack of increase in molecular concentration found in this autolysis was due to the phosphate solution on account of its alkalinity (phenolphthalein) and this fact agrees with observations of previous investigators that alkalies inhibit autolysis. An alkalinity equivalent to this never occurs in the body.

In one experiment (29D) where dihydrogen sodium phosphate served as diluent, the autolytic mixture was strongly acid to phenolphthalein. This experiment should be compared with Experiment

TABLE VI.[17]

	Control.	2 Hours.	4 Hours.	6 Hours.	8 Hours.	10 Hours.
Exp. 29D	0.635	0.635	0.680	0.725	0.765	0.795
Washed; not fed; 5	5.05	5.30	5.50	7.40	12.40	14.35
per cent. H_2NaPO_4	10.95	11.7	12.85	13.05	13.50	14.05
	1.85	1.75	1.95	1.75	1.60	1.65
Exp. 33C	0.685	0.725	0.745	0.820	—	1.285
Unwashed; not fed; 1	3.25	4.50	7.40	14.90	—	33.85
c.c. ethyl butyrate	1.10	1.30	1.85	3.00	—	5.10
with saline diluent	2.00	2.10	2.45	2.80	—	8.60
Exp. 36C	0.810	0.815	0.845	0.920		1.515
Unwashed; fed; 1 c.c.	5.80	5.60	5.35	10.05		28.15
ethyl butyrate with	1.25	1.55	1.60	2.35		6.65
saline solution	1.10	1.55	1.45	1.65		5.35
Exp. 27E	0.625	0.650	0.670		0.705	
Unwashed; not fed;	6.25	6.10	6.10		6.50	
warm Locke's solu-	0.40	0.60	0.80		1.00	
tion	0.75	0.70	0.70		1.00	
Exp. 31A	0.630	0.720	0.775		0.800	1.015
Unwashed; well-fed;	5.60	5.65	5.65		7.20	20.80
warm Locke's solu-	0.60	0.75	0.85		1.15	6.80
tion	0.40	0.75	0.75		0.85	2.75

[17] The upper left-hand figures represent degrees of depression of the freezing-point; the upper right-hand figures, non-coagulable nitrogen expressed as cubic centimeters of *N*/10 acid; the lower left-hand figures, acidity to phenolphthalein in terms of cubic centimeters of *N*/10 acid; lower right-hand figures, alkalinity to dimethylaminoazobenzene in terms of cubic centimeters of *N*/10 alkali.

29E (Table II, Series IV) and the control with saline. No noticeable variation in rate of autolysis can be detected.

In Experiments 33 and 36 in which pure ethyl butyrate was added to the autolytic mixture a most marked increase in acidity (phenolphthalein) was observed together with the greatest augmentation of non-coagulable nitrogen and the depression of the freezing-point obtained in any of the experiments. These two experiments ran for twelve hours and were absolutely sterile throughout. The depression of the freezing-point increased in Experiment 36 from 0.810° to 1.515° and in Experiment 33 from 0.685° to 1.285°. The non-coagulable nitrogen rose in Experiment 36 from 5.8 to 28.15 and in Experiment 33 from 3.25 to 33.85[18]. Considered from every standpoint the autolysis was most strikingly accelerated by the formation of the butyric acid during the progress of the autolysis.

In general, therefore, attempts to alter the rate of autolysis by means of acid and alkaline phosphates and bicarbonates resulted negatively. Some evidence was obtained that an alkaline reaction tends to inhibit the nitrogenous autolysis; this was especially well marked in Experiment 28C. The production of butyric acid during autolysis set up a marked acceleration in the rate of the decomposition.

Salt Concentration.—It seemed plausible that one of the conditions which favored the onset of autolysis might consist in the decrease in concentration of the salts (mainly calcium and potassium compounds) of the tissue as the result of the dilution with sodium chloride and phosphates. Such a condition would, of course, be accentuated in a perfused organ since during the irrigation the calcium, potassium, and other salts would diffuse out from the cell into the perfusing solution.

In order to partially avoid such diminished concentration, in two experiments (27E and 31A) Locke's[19] solution was added as dilu-

[18] In Experiment 36 it is noticeable that the acidity did not increase very rapidly during the first four hours and that the nitrogenous autolysis suffered a latent period.

[19] When physiological saline is employed as diluent, the osmotic concentration of the tissues is supposed to continue the same as when the blood is circulating through the organ. The percentage concentration of the salts other than sodium chloride is nevertheless markedly diminished. Locke's solution which approaches the blood as regards its content of inorganic salts was utilized for this reason.

ent and the autolytic rate studied. Although the results show an increase in ionic concentration, the figures for the non-coagulable nitrogen are plainly indicative of a lack of autolysis existing up to the fifth hour. While one cannot draw too definite conclusions from the results of only two experiments, it seems reasonable to believe that the retaining of the salt content of the tissue in approximately unchanged concentration, acts as another factor tending to inhibit or at least to retard nitrogenous autolysis. It is also probable that the Ca or K ions are in some way active in this regard, and that the difference in rate of autolysis noticed between washed and unwashed organs can be ascribed to this cause in part.

This question was attacked in another way. The tissues were aseptically removed and allowed to autolyze *en masse* in the thermostat and then diluted as usual with three volumes of physiological saline. Unfortunately no figures for non-coagulable nitrogen were obtained in this series[20].

The depression of the freezing-point for the tissue itself and the diluted mixture increased at about the same rate as in all other experiments.

DISCUSSION.

The two procedures employed for the determination of the inception and rate of autolysis have been utilized previously by several investigators. The increase in non-coagulable nitrogen was the first test applied in this direction since it was early recognized that in the autolytic hydrolysis the coagulable nitrogen of the protein became changed into nitrogen of substances which were of smaller molecular weight and could not be rendered insoluble by heat. More recently, however, the attempt to control this procedure by other methods has resulted in the determination of the electrical conductivity and of the molecular or ionic concentrations of the solutions undergoing autolysis. If we are dealing with autolytic mixtures of pure protein, hydrolytic changes occurring in the solution would make themselves evident by all three methods, but not

[20] For this reason the study of this type of experiment is to be continued more particularly with reference to possible differences in the rate of autolysis after the organ has been prepared by perfusion *in situ* with solutions of various types of salts, and in this way the attempt may be made to alter the conditions existing in the cell before the commencement of autolysis.

simultaneously, since in the initial stage of the reaction the proteins would become transformed into proteoses or non-coagulable nitrogen, without any decided augmentation in molecular or ionic concentration sufficient to effect the depression of the freezing-point or electrical conductivity. Toward the end of autolysis the maximum proteose-nitrogen might be reached while progressive hydrolysis would continue to break down the molecules still further with consequent increased molecular concentration thereby affecting the depression of the freezing-point. The electrical conductivity on the other hand could only be increased by the formation of ionized molecules. From this it is evident that even in a simple solution of protein these three factors might not run parallel.

In the cell, however, the process becomes still more complex. Autolysis of non-nitrogenous substances such as glycogen, the lipoids, neutral fats and the rearrangement of inorganic salts would set up changes in molecular and ionic concentration which would not be indicated by a study of variations in non-coagulable nitrogen. It is hard, therefore, to agree with the statement of Wells that determinations made of these three factors (non-coagulable nitrogen, depression of the freezing-point and electrical conductivity) paralleled one another. That they might do so must be admitted; that the chances are very much against their doing so is most probable.

A general survey of the results obtained in this investigation presents much evidence in favor of the latter view. In some experiments (5b, 10, 22, 23, 27E and 28A and 28C) conclusions would be reached from the figures obtained from the non-coagulable nitrogen that no autolysis had occurred up to the sixth or eighth hour. In fact in Experiments 28A and 28C, less non-coagulable nitrogen was present in solution at the end of seven and one-half hours than in the control at the outset of the experiments. One could naturally assume that non-coagulable nitrogen had become synthetized into protein nitrogen. The figures for the depression of the freezing-point, however, show that the molecular or ionic concentration was increasing gradually up to the end of the experiments. This is especially well marked in Experiment 28C, where the depression of the freezing-point increased from 0.680° (control) to 0.805° (seven and one-half hours). Evidently non-nitrogenous molecules were

undergoing hydrolytic cleavage with the production of smaller and more numerous molecules.

We do not have to look far for an explanation of these results. It has been mentioned elsewhere that with apparently the same conditions of diet, the liver of dogs varies markedly in the content of fat and of glycogen and there is also variation in the rapidity with which the latter disappears. With these points in mind a study of the notes taken in various experiments plainly brings out the fact that in those cases where the glycogen disappeared most rapidly as deduced by the decrease in opalescence of the filtrate after heat coagulation, the depression of the freezing-point tended to increase regardless of changes in non-coagulable nitrogen. In the absence of glycogen the depression of the freezing-point was not affected in this manner. Evidently the increased depression of the freezing-point occurred in greatest part at the expense of the hydrolysis of glycogen, the latter being transformed into smaller molecular compounds, such as maltose, lactic acid, etc.

Concerning lipolytic changes, it becomes more difficult to determine what part these processes are playing in the whole general autolytic change, since there is no way of visually following the transformation as is the case with glycogen. Increased acidity in the autolytic mixture may be the result of the hydrolysis of fats or equally well of the carbohydrates. It is, therefore, of the utmost difficulty to decide to what process is due the increased acidity as determined by phenolphthalein. From the fact that in a majority of the experiments there occurred a gradual increase in acidity (phenolphthalein) associated with no simultaneous changes in reaction to dimethylaminoazobenzene, it seems reasonable to consider that the augmented acidity was not brought about by increase in acid phosphates since dimethylaminoazobenzene would react to such change; but rather to a formation of acids of the fatty acid type to which the latter indicator is not sensitive.

In this connection emphasis must be laid upon the results of the two experiments in which ethyl butyrate was added to the autolytic mixture. The pronounced acceleration of autolysis which occurred evidently as the result of the formation of butyric acid in the mixture cannot fail to have but one significance. Given

a cell in which as the result of increased lipolysis from some reason or other fatty acids are being produced in excess, their presence will markedly stimulate nitrogenous autolysis. The fat and fatty acids content of supposedly normal cells vary within very wide limits. This is a factor which must be reckoned with in the consideration of general or even nitrogenous autolysis. Whether the augmented autolysis is a result of the catalytic action of the hydrogen ions of the butyric acid or whether the same relationship exists between the lipolytic autolyses and the decomposition of protein substances, is hard to decide.

From this somewhat cursory discussion it becomes evident that at the time when the cell becomes removed from its circulation, hydrolytic changes are set up with varying degrees of rapidity; the character of these changes is dependent upon the condition of the cell at the time. The reaction of the fluids bathing the protoplasm (if the protoplasm is considered to be neutral) is an important factor as shown by the many investigations upon the effect of acidity and alkalinity upon autolysis. The results recorded here plainly indicate however that changes in reaction which most nearly simulate those which can occur in the living cell do not alter the autolytic rate so markedly, if they do at all, as where stronger acids and alkalies are employed.

In the majority of cases only the nitrogenous autolysis has been studied. As it has been indicated, if carbohydrates are present in the cell, amylolytic autolysis will also occur; lipolytic autolysis probably is as constant as the nitrogenous process. Either one of the three types of autolysis may occur without the other. Probably all three originate when general autolysis begins. That one process influences the others and that the products of the autolytic hydrolyses (lactic acid, fatty acids, etc.) markedly influence, perhaps augment the others (*e. g.*, nitrogenous autolysis) are facts rapidly being brought into prominence. Further study in this direction should lie in attempts to test the effect upon autolysis of a single type, *e. g.*, nitrogenous, by setting up simultaneously in the mixture amylolytic or lipolytic autolysis; this would also include the effect of autolytic products, upon the rate of all three types of autolysis.

CONCLUSIONS.

1. Under certain conditions, general autolysis does not begin immediately upon the removal of the organ from its circulation. This latent period is more apt to be present in those cases in which the tissues have been temporarily cooled on account of the use of a cold saline diluent or in which the percentage concentration of the inorganic salts (calcium or potassium), of the tissues have been changed by dilution with a sodium chloride solution. The presence of blood and absence of fats and of glycogen in the cells act as important factors in prolonging the latent period.

2. Attempts to produce an alkaline reaction (phenolphthalein) in the tissue resulted negatively. Solutions of disodium hydrogen phosphate and of sodium bicarbonate when added to the liver tissues gave a mixture which was acid to phenolphthalein and had no apparent effect upon autolysis.

3. The addition of antiseptics—chloroform and toluol—markedly decreased the rate of autolysis. Ordinary light produced no effect.

4. Ethyl butyrate when added to the tissue became hydrolysed into butyric acid; the formation of this acid in the mixture caused a decided acceleration in the autolytic rate. The acidity of a solution of dihydrogen sodium phosphate failed to produce a similar result.

5. The figures for the changes in the depression of the freezing-point, non-coaguable nitrogen and reaction of the autolytic mixture do not parallel one another. In some experiments a marked increase in the depression of the freezing-point was unaccompanied by augmentation of non-coagulable nitrogen.

6. General autolysis is the sum total of proteolytic, amylolytic and lipolytic factors. Each of these autolytic factors may proceed alone for a time; the rate of one is decidedly influenced by the presence or absence of the others. The acid products which are the result of amylolytic (lactic acid) and of lipolytic (higher fatty acids) autolysis, exert a pronounced augmentative effect upon the commencement and rate of nitrogenous autolysis.

BIBLIOGRAPHY.

1. Lane-Claypon, J. E., and Schryver, S. B., *Jour. of Physiol.*, 1904, xxxi, 169.
2. Schryver, S. B., *Biochem. Journal*, 1906, i, 123.
3. Wiener, H., *Centralbl. f. Physiol.*, 1905, xix, 349.
4. Henderson, L. J., and Black, O. F., *Amer. Jour. of Physiol.*, 1907, xviii, 250.
5. Fredericq, *Bull. de l'Acad. royale de Med., Belg. Séance* du 29 Nov., 1902, xvi, 699.
6. Delrez, L., *Arch. internat. de physiol.*, 1904, i, 152.
7. Wells, H. G., and Benson, R. L., *Jour. of Biol. Chem.*, 1907, iii, 35; cf. also Liagre, Ch., *Arch. internat. de physiol.*, 1904, i, 172.
8. Magnus-Levy, *Hofmeister's Beiträge*, 1902, ii, 261.
9. Schryver, S. B., *loc. cit.*
10. Palladin, W., *Zeitschr. f. physiol. Chem.*, 1908, lv, 207.
11. Baer, J., *Arch. f. exp. Path. u. Pharm.*, 1906, lvi, 68; Bear, J., and Loeb, A., *ibid.*, 1905, liii, 1; Pearce, R. M., and Jackson, H. C., *Jour. of Exper. Med.*, 1907, ix, 550.
12. Jackson, H. C., and Pearce, R. M., *Jour. of Exper. Med.*, 1907, ix, 520.
13. Seitz, W., *Arch. f. ges. Physiol.*, 1906, cxi, 309.
14. Lang, S., *Beiträge z. chem. Physiol. u. Path.*, 1907, iii, 35.
15. Cf. v. Dryjewezki, A., *Biochem. Zeitschr.*, 1906, i, 229; Wiener, H., *loc. cit.*; Baer, J., *loc. cit.*; Baer, J., and Loeb, A., *loc. cit.*

THE RELATION OF PROTEIN, LIPOIDS AND SALTS TO THE WASSERMANN REACTION.[1]

By HIDEYO NOGUCHI, M.D.

(*From the Rockefeller Institute for Medical Research, New York.*)

In the present work on the Wassermann reaction in syphilis and general paralysis several points have been especially considered, namely: (1) the quantitative estimation of the active substances in syphilitic blood sera and paralytic cerebro-spinal fluids; (2) the effects of physical and chemical agents upon the active substances; (3) the quantitative estimation of the globulin content of syphilitic blood sera and paralytic spinal fluids; (4) the alcohol-extractibility of the active substances of syphilitic sera and paralytic spinal fluids; and (5) the antigenic lipoids and salts entering into Wassermann's reaction.

THE QUANTITATIVE ESTIMATION OF THE ACTIVE SUBSTANCES OF SYPHILITIC BLOOD SERA AND PARALYTIC CEREBRO-SPINAL FLUIDS.

The blood sera from fifty-five syphilitic patients have been studied with the view of determining the minimal quantity of serum just sufficing to produce complete fixation[2] of complement, the amount of which was contained uniformly throughout the entire work in 0.1 c.c. of fresh serum of the guinea-pig. Of eight cases of primary syphilis, five gave positive reactions, the titers ranging from 0.1 to a little over 0.2 c.c. The majority of the titers were 0.2 c.c. Of forty-two cases of secondary syphilis, six have been untreated and gave positive reactions in titers of 0.0006, 0.002, 0.003, 0.004, 0.01 and 0.04 c.c. respectively. Seventeen cases had

[1] Received for publication September 1, 1908.

[2] In each tube 3 hæmolytic units of anti-sheep amboceptor (rabbit serum) were employed, the quantity being 0.003 c.c. The alcoholic extracts of the blood and liver of man or rabbits were used as "antigens" in quantities of 0.1 to 0.2 c.c. of a 0.1 per cent. or 0.2 per cent. suspension. Purified preparations of lecithin were also employed as "antigens."

been slightly treated and gave positive reactions in titers of 0.01 to 0.2 c.c., or more frequently of 0.02 to 0.06 c.c. This group and the group of untreated cases of secondary syphilis gave the highest titers of all the cases examined. Seven cases were moderately treated and gave positive reactions in titers of 0.2 or a little more than 0.2 c.c. Twelve cases were in the late secondary stage under steady and prolonged treatment, of which only two gave positive reactions in a titer weaker than 0.2 c.c. The remaining ten cases gave negative results. Five cases of tertiary syphilis, which had been under steady and prolonged treatment, gave negative reactions excepting one in which the test was positive and the patient had lesions of the nervous and osseous systems.

Thirty-four control sera were examined; 11 were from normal persons and were uniformly negative; 23 were from persons suffering with other diseases than syphilis; of these five reacted positively in titers from 0.05 to 0.2 c.c. I could not exclude absolutely a syphilitic infection in the latter.

The cerebro-spinal fluid from cases of general paralysis gave in the majority of instances titer of 0.1 to 0.2 c.c. It was rarely that a sample with a titer of 0.02 c.c. was met with. I have not met an instance in which 0.01 c.c. produced complete inhibition of hæmolysis.

Hence the highest titer of any syphilitic serum was 0.0006 c.c., and of any cerebro-spinal fluid, 0.02 c.c.

THE EFFECTS OF PHYSICAL AND CHEMICAL AGENTS UPON THE ACTIVE SUBSTANCES.

The active substances of unfractionated syphilitic serum or of the globulin fractions obtained by half-saturation with ammonium sulphate, or through dialysis, are inactivated at 70° to 76° C. in twenty minutes. I may add that the activity of the euglobulin fraction obtained through dialysis disappears more easily when heated in distilled water than when heated in 0.9 per cent. saline solution. It was almost completely destroyed by a temperature of 65° C.

Drying syphilitic serum at 24° C. does not affect its activity in regard to the Wassermann reaction, and heating in a dry state to

temperatures below 100° C. for twenty minutes leaves its activity unimpaired. The temperatures of 120° and 135° C. produce partial insolubility of the dry serum and render it almost inactive.

The cerebro-spinal fluids from cases of general paralysis were, in my experience, which agrees with the findings of Marie and Levaditi,[3] rendered inactive in ten to twenty minutes by temperatures of 70° to 80° C.

Concerning the deterioration of the active substances with age, I may state that a sample of serum which gave a titer of 0.004 c.c. after the first twenty-four hours was reduced to a titer of 0.01 c.c. after fifty-six days in the refrigerator at 2° C. Some spinal fluids obtained from cases of general paralysis and almost a year old gave a titer of 0.1 to 0.2 c.c., no quantitative observations having been made when they were first collected. Bruck and Stern[4] state that the active principle in syphilitic sera will retain its strength with little deterioration when kept in the refrigerator, no length of time being assigned.

The activity of cerebro-spinal fluids from patients suffering from general paralysis is destroyed by acids or alkalies. When 0.2 c.c. of a fairly active sample of spinal fluid of a paralytic is mixed with 1.6 c.c. of a 0.9 per cent salt solution with an acidity or an alkalinity corresponding to $N/40$ concentration, the fluid becomes so modified that after removal of acid (HCl) or alkali (NaOH) by neutralization, at the end of one hour at 37° C., it no longer gives the Wassermann reaction. A concentration above $N/40$ of hydrochloric acid or sodium hydroxide in the same amount causes marked reduction of hæmolysis. This antihæmolytic phenomenon may possibly be due simply to increase in the salt tonicity after the neutralization.

Trypsin (Grübler's Trypsin sicc.) in neutral or a weakly alkaline (0.5 per cent. Na_2CO_3) medium caused complete inactivation of the syphilitic sera or cerebrospinal fluids. The ferment was allowed to act at 37° C. for three hours before the tests were made. It was customary to subject 0.2 c.c. of the serum or spinal fluid to the digestion and then to test the entire mixture, after neutralization in case of alkaline reaction, for the Wassermann reaction.

[3] Marie and Levaditi, *Annales de l'Inst. Pasteur*, 1907, xxi, 138.
[4] Bruck and Stern, *Berl. klin. Woch.*, 1908, xlv, 301.

Pepsin (Grübler's Pepsin puriss.) was found to be without effect when added to the serum or spinal fluids of syphilitics and paralytics in neutral medium. In the presence of a 0.2 per cent. hydrochloric acid it destroyed the activity of these fluids very promptly and completely. The acid alone had been found to be injurious when used in that strength, although a more complete disappearance of the active substances in the presence of pepsin conclusively demonstrated the effect of this ferment. It seems to be of some interest to recall here that, in spite of the pronounced antienzymotic properties of blood serum, the active substances of the serum and spinal fluid of syphilitics and paralytics are easily destroyed by tryptic and peptic digestion.

The activity of the syphilitic sera and paralytic cerebro-spinal fluids disappears when they are exposed to the combined effects of a photodynamic substance and direct sunlight. Eosin "rein" in dilution of 0.03 per cent. destroys the substances within three hours in the presence of direct sunlight. No visible precipitation occurs in the exposed eosinized mixtures. A three hours' exposure of non-eosinized controls to direct sunlight produces a perceptible diminution of the activity. On the other hand, no deteriorating effect is exerted by the dye in the dark. Whether the destructive action of eosin in direct sunlight is, in part or *in toto*, due to the acid formation in the mixture has not been determined.

THE QUANTITATIVE ESTIMATION OF THE GLOBULIN CONTENT OF SYPHILITIC SERA AND PARALYTIC CEREBRO-SPINAL FLUIDS.

Klausner[5] found that by adding adequate quantities of distilled water to syphilitic sera a precipitate would form, and Sachs and Altmann[6] observed a similar precipitate when a weak alcohol is employed instead of distilled water. The differences between syphilitic and non-syphilitic sera in these two tests are quantitative and become indistinguishable upon prolonged standing. Klausner thought his reaction due to an increase in globulins in syphilitic sera. Porges and Meier[7, 8] discovered that the sera of syphilitic patients

[5] Klausner, *Wien. klin. Woch.*, 1908, xxi, 214, 363.
[6] Sachs and Altmann, *Berl. klin. Woch.*, 1908, xlv, 522.
[7] Wassermann, *Berl. klin. Woch.*, 1907, xliv, 1599, 1634.
[8] Porges, *Wien. klin. Woch.*, 1908, xxi, 206.

can be differentiated from normal sera by the appearance of a quicker and more abundant precipitation of lecithin emulsion upon addition of these sera.

The diagnostic value of the Wassermann reaction and the Porges-Meier reaction for syphilis has been found to be, to a great extent, similar. Considering the rôle which lecithin plays in both reactions, it would seem not improbable that the active substances entering into these reactions are identical. Many chemicals to which antigenic properties had been assigned are also found to produce the precipitation phenomenon with syphilitic fluids. Thus sodium oleate and sodium glycocholate were shown by Nobl and Arzt[9] and Fritz and Kren[10] to give a similar reaction to lecithin. According to Elias, Neubauer, Porges and Salomon[11] the active substances belong to globulins and are present both in normal and syphilitic sera, differing only in the quantities which are greater in the latter than in the former.

No quantitative estimation of the serum globulin content of the syphilitic sera has yet been systematically made.

The presence in the spinal fluid of general paralytics and tabetics of the active substances has repeatedly been shown by previous workers.[12, 13, 14] Long before the Wassermann reaction had been applied to these cases an increase in proteins in the cerebro-spinal fluid had been known to be characteristic to these diseases.[15]

In my present investigations thirty-three cases of syphilis and twenty cases of other diseases than syphilis, including five normal individuals, have been subjected to simultaneous tests for the globulin content and for the Wassermann reaction. The method of estimating the quantity of serum globulins was a relative one and consisted of weighing the precipitate obtained by half-saturation of the sera with ammonium sulphate (or 1 part of serum + 4 parts of a

[9] Nobl and Arzt, *Wien. klin. Woch.*, 1908, xxi, 287.
[10] Fritz and Kren, *Wien. klin. Woch.*, 1903, xxi, 386.
[11] Elias, Neubauer, Porges and Salomon, *Wien. klin. Woch.*, 1908, xxi, 748, 831.
[12] Wassermann and Plaut, *Deutsch. med. Woch.*, 1906, xxxii, 1769.
[13] Marie and Levaditi, *Annales de l'Inst. Pasteur*, 1907, xxi, 138.
[14] Morgenroth and Sterz, *Virchow's Archiv*, 1907, clxxxviii, 166.
[15] The serum must not contain hæmoglobin.

half-saturated solution of $(NH_4)_2SO_4$). The precipitate was first centrifugalized at a rate of 5,000 revolutions per minute, during twenty minutes, decanted, the clear supernatant fluid poured off, the residual moisture absorbed with soft filter paper, and the solid parts then weighed in the centrifuge tube, whose own weight had been previously known. Beside this direct estimation, I found the following indirect method to be of some use in avoiding tedious weighing of the globulin deposit. This indirect estimation is based upon the precipitation of serum globulins by weak solution of acids. Take 1 c.c. of serum and mix it with 4 c.c. of half-saturated solution of ammonium sulphate, centrifugalize, pour the supernatant fluid away, then dissolve the deposit (globulins) in 10 c.c. of 0.9 per cent. salt solution. The clear globulin solution so prepared is then mixed with an equal volume of 10–15 per cent. butyric acid[16] solution containing 0.9 per cent. sodium chloride, shaken well and left at room temperature. The test is observed from time to time. With the globulin solution from normal serum there is, as a rule, no, or at most, slight opalescence even at the end of two hours from the time of acidification. With the globulin solution from syphilitic serum, which gives positive Wassermann test, a dense cloudiness arises promptly and within thirty minutes or later it becomes flocculent, and finally a deposit in the bottom of the test tube occurs. In the following table some of my experiments on this point are given. The ciphers given under the column "Weight of serum-globulins" denote the weight of the moist deposit of the globulins precipitated from 1 c.c. of serum and approximately correspond with eight to nine times the real weight as obtained by proper processes (coagulation with alcohol, hot water, removal of salts, extraction of fatty substances, and drying to constant weight).

The foregoing experiments show that in the blood sera of untreated or slightly treated cases of primary and secondary syphilis there is a definite increase in the globulin content. The results obtained by means of the direct and indirect methods of globulin estimation indicate that an increase in the globulin content is paralleled by the positive results of the Wasserman test, the only difference be-

[16] Various acids other than butyric have been found to be less satisfactory for this purpose.

tween the two being the slightly higher percentage of positive results with the indirect globulin method. There was no quantitative parallelism between the globulin content and the titers of the active substances of syphilitic sera. A syphilitic serum can have a low titer or be active for the Wassermann reaction without showing a correspondingly high globulin content. Among the controls the sera from the normal individuals and gonorrheal patients gave in-

TABLE I.

Syphilitic Sera.

	Stage.	Treatment.	Direct Estimation. Weight of the Globulins Weighed as Moist Deposit.	Indirect Estimation. Flocculent Precipitation of Globulin Solution with 10 per cent. Butyric Acid.	Wassermann Reaction.
1	I	o	0.348 gram	++	+ > 0.2 c.c.
2	"	o	0.321	++	+ 0.2
3	"	o	0.355	++	+ 0.2
4	"	o	0.280	+	−
5	"	×	0.200	−	−
6	II	o	0.553 (!)	++++	+ 0.04
7	"	×	0.395	+++	+ 0.02
8	"	×	0.260	+	+ 0.2
9	"	×	0.250	+	+ > 0.2
10	"	×××	0.257	+	−
11	"	×	0.411	++++	+ 0.02
12	"	×	0.402	+++	+ 0.2
13	"	×	0.353	++	+ 0.05
14	"	×	0.279	+	+ > 0.2
15	"	×	0.300	++	+ > 0.2
16	"	××	0.425	+++	+ 0.2
17	"	××	0.298	++	+ 0.1
18	"	××	0.365	+++	+ 0.2
19	"	××	0.393	++	+ > 0.2
20	"	×××	0.251	−	−
21	"	×××	0.343	+++	+ > 0.2
22	"	×××	0.161	−	−
23	"	×××	0.149	−	−
24	"	×××	0.200	−	−
25	"	×××	0.141	−	−
26	"	×××	0.210	−	−
27	"	×××	0.140	−	−
28	"	×××	0.253	+	−
29	"	×××	0.230	+	−
30	"	×××	0.252	+	−
31	III	×	0.450	++++	+ 0.1
32	"	××××	0.110	−	−
33	"	××××	0.105 (!)	−	−

Under column "Treatment" o = no treatment, × = slight treatment, ×× = moderate treatment, ××× = much treatment, ×××× = treatment extending over two years.

TABLE II.

Non-syphilitic[17] *and Normal Sera.*

Nature of Disease.	Direct Estimation of Globulins.	Indirect Estimation of Globulins.	Wassermann Reaction with the Serum.
Gonorrhœa.	0.205	—	—
"	0.135	—	—
"	0.175	—	—
"	0.130	—	—
"	0.110	—	—
"	0.140	—	—
Rheumatic fever.	0.183	—	—
Gastric ulcer.	0.170	—	—
Cirrhosis of liver.	0.350	+++	+ 0.05 c.c.
Chronic valvular disease, etc.	0.305	++	+ 0.2
Tuberculosis.	0.283	+	+ 0.2
Pneumonia lobar.	0.166	—	—
Chronic interstitial nephritis.	0.251	—	—
Malaria.	0.312	++	—
Tuberculosis.	0.321	++	+ 0.05
Normal.	0.165	—	—
"	0.163	—	—
"	0.184	—	—
"	0.205	—	—
"	0.204	—	—

variably negative results and no increase in or abnormally large globulin content could be found by direct estimation. Of the rest of the controls four sera showed an increase of globulin and reacted positively to the Wassermann test.

My experiments on cerebro-spinal fluids from forty-three cases of general paralysis[18] showed that there is a closer relation between the increase of leucocytes and that of globulins than between the increase of globulins and the positive reaction to the Wassermann test. In other words, the results obtained by cytological diagnosis and globulin estimation are in good harmony, while the Wassermann reaction was in some instances absent although the globulin was increased. In forty-three cases, thirty-seven positive results were obtained. In two of forty control cases, where the globulin tests were all negative, the Wassermann reaction was faintly positive.

[17] The possibility of a previous syphilitic infection could not be excluded in some of the sera giving the positive reaction for the Wassermann test.

[18] A fuller account of this series and of still other cases will be published elsewhere in conjunction with Dr. J. W. Moore.

The method which I employed for detecting an increase of the protein in the cerebro-spinal fluids may be mentioned here, as it affords a much sharper and more enduring means to differentiate normal from paralytic spinal fluid than the Nonne method of half-saturation with ammonium sulphate.[19] It is very simple and can be made with small quantities of material in less than half an hour. Its diagnostic value has been found to be equivalent, if not superior, to that of cytological examination. The technic is as follows:

To 0.1 c.c. of spinal fluid[20] add 0.5 c.c. of the 10 per cent. butyric acid,[21] boil briefly over a flame, then add quickly 0.1 c.c. of normal solution of sodium hydroxide and reheat briefly. Allow the test tube to stand in a frame and observe the reaction. Read the reaction for diagnosis within three hours; longer standing, extending to twenty-four hours, offers no advantage, and the characteristic appearance of the reaction becomes less obvious after the lapse of this time. In the spinal fluid from cases of general paralysis *a coarse granular or flocculent precipitate* appears, which gradually settles down to the bottom of the test-tube leaving the fluid above clear. In the majority of cases of general paralysis, tabes dorsalis and cerebro-spinal lues, the precipitate becomes granular within ten to twenty minutes and settles in a short time afterwards. In the spinal fluid from cases of alcoholic psychosis, dementia præcox, and epilepsy as well as from normal subjects, there occurs *a slight and uniform opalescence* only, and no coarse precipitate forms even after several hours. In case of an ambiguous reaction a test with 0.2 c.c. of the spinal fluid must be made which usually decides the point.

In determining the quantities of proteins, especially those precipitable by half-saturation of ammonium sulphate in cerebro-spinal

[19] This method consists of mixing 2 c.c. of spinal fluid with an equal volume of saturated solution of ammonium sulphate (strictly neutral) and reading the result within three minutes. The differentiating value gradually diminishes as time elapses beyond three minutes.

[20] Specimens which had been preserved in the refrigerator for about one year, or those from which all cellular elements have previously been removed by centrifugalization, are equally good for the test. It is entirely to one's individual taste whether one or a multiple of the quantities indicated above will have to be employed.

[21] A number of inorganic and organic acids have been tried, but were found to be less satisfactory.

fluid, I found that the ones which are peculiarly rich in the paralytic spinal fluid, are chiefly globulins or globulin-like substances which are the main substances causing the characteristic coarse or flocculent precipitate in the above test as well as the ones entering into the Wassermann reaction. The average quantities of these proteins contained in 1 c.c. of fluid ranged from 0.01 to 0.02 gram[22] weighed as a moist deposit of constant density. A similar estimation with non-paralytic[23] and normal spinal fluids, showed that the amount of the protein never exceeded one-tenth of that found in the specimens from paralytics.

CAN THE ACTIVE SUBSTANCES OF SYPHILITIC SERA AND PARALYTIC CEREBRO-SPINAL FLUIDS BE EXTRACTED?

Levaditi and Yamanouchi[24] claim that the reacting substances of syphilitic sera and paralytic and tabetic spinal fluids can be extracted with alcohol. By alcoholic extraction they obtained a certain amount of lipoids and of salts. Both sets of substances were inherently antihæmolytic when used in large enough quantity. Mixed in smaller quantity with liver extract or other "syphilis antigens" they gave increased inhibition of hæmolysis. Apparently Levaditi and Yamanouchi consider the Wasserman reactions as a summation of the anticomplementary effect of two sets of lipoids and salts. They give no quantitative details.

My own experiments in this respect failed to convince me that the Wassermann reaction is caused by alcohol-soluble constituents of serum and spinal fluid.

By means of dialysis of the serum in a celloidin sac the englobulin fraction of the serum was obtained free from dialysable salts. The euglobulin fractions were redissolved in 0.9 per cent. salt solution and tested for the Wasserman reaction. Three samples of syphilitic and two samples of normal sera examined showed that the euglobulin obtained from syphilitic sera possesses the property of giving the Wassermann reaction while that from normal sera did not. The titers of the globulin fractions were the same as those of the corresponding sera.

[22] To obtain an approximate absolute weight the ciphers must be divided by 8 to 9.

[23] Cases of tabes dorsalis are not included.

[24] Levaditi and Yamanouchi, *Compt. rend. de la Soc. de Biol.*, 1908, lxiv, 27.

The above experiment shows that the active substances binding complement with lipoids remain in the celloidin sac on dialysis. The removal of dialysable salts from the serum does not affect the activity of the specific substances which are precipitated with the euglobulin fraction. A qualitative difference is shown to exist between normal and syphilitic serum-globulins.

The lipoidal bodies of syphilitic sera and paralytic spinal fluids are alleged by Levaditi and Yamanouchi[25] to represent the specific active syphilitic substance of these fluids. In studying this question I have undertaken numerous quantitative estimations of alcohol-extractable constituents of blood sera, blood coagula and spinal fluids of normal, syphilitic and paralytic subjects, in order to ascertain if there exist qualitative or quantitative difference between the lipoids derived from normal and from syphilitic materials. Two syphilitic sera and one normal serum were extracted with ten volumes of 95 per cent. alcohol for five days at 37° C. The weights of the alcohol soluble matters were, after drying, 0.205, 0.210 and 0.202 gram, respectively, per 11.5 c.c. of the serum (ca. 1.79 per cent. average). The sample which yielded 0.205 gram of alcohol-extractable substances had a titer of 0.004 c.c., that which yielded 0.210 gram had a titer of 0.01 c.c., and that which yielded 0.202 gram was normal and had no effect on complement in Wassermann's test. The alcoholic extracts were dissolved in 0.9 per cent. saline solution (11.5 c.c., the original volume of serum) respectively, and tested for the property of "syphilitic antibody." When used in quantities over 0.3 c.c. these solutions were slightly antihæmolytic, but there was no regular complement fixation even when used in combination with antigenic substances (lecithin, alcoholic extract of liver, etc.). There was no difference between the solutions obtained from normal and from syphilitic sera, both being entirely devoid of the "antibody" property which characterizes the whole syphilitic sera.

Three samples of blood coagula of syphilitic subjects (manifest secondary stage) and three of normal persons were extracted with ten volumes of 95 per cent. alcohol at 37° C. for five days. The quantities of the coagula and of the lipoids and salts extracted with alcohol may be stated as follows:

[25] *Loc. cit.*

Syphilitic coagula I	20 grams.	Extract 0.230 gram.
II	17 grams.	Extract 0.219 gram.
III	15 grams.	Extract 0.190 gram.
Normal coagula I	32 grams.	Extract 0.330 gram.
II	20 grams.	Extract 0.210 gram.
III	28 grams.	Extract 0.299 gram.

The average content of syphilitic and normal blood coagula in alcohol-extractable matters is approximately 1.1 per cent., of which salts make up a large percentage.

The experiments in regard to the existence of specific active syphilitic substance in these extracts have been negative. No positive reaction for the Wassermann test was obtained with any of them.

Similar extraction experiments were made with three samples of general paralytic spinal fluids and one of a non-paralytic patient. None of the extracts gave a positive Wassermann test conclusive enough for establishing a "syphilitic antibody" nature. In a similar way to Levaditi and Yamanouchi, I observed in one of the paralytic extracts more or less marked inhibition of hæmolysis in combination with lecithin, but I am not convinced that this reaction is to be regarded as the regular Wassermann reaction.

ANTIGENIC LIPOIDS AND SALTS FOR WASSERMANN'S REACTION.

Landsteiner, Müller and Plötzl[26] discovered the alcohol solubility of the antigenic principles of syphilitic and normal tissues. Porges and Meier[27, 28] found lecithin to act as antigen, while Levaditi and Yamanouchi[29] place sodium glycocholate, sodium taurocholate, protagon and cholin among the syphilitic "antigens," and found lecithin to be less active than sodium glycocholate. Sachs and Altmann[30] state that sodium oleate has a similar action. Cholesterin and vaselin were effective as antigens in the hands of Fleisch-

[26] Landsteiner, Müller and Plötzl, *Wien. klin. Woch.*, 1907, xx, 1421, 1565.
[27] Wassermann, *Berl. klin. Woch.*, 1907, xliv, 1599, 1634.
[28] Porges, *Wien. klin. Woch.*, 1908, xxi, 206.
[29] Levaditi and Yamanouchi, *Compt. rend. de la Soc. de Biol.*, 1907, lxiii, 740; 1908, lxiv, 349.
[30] Sachs and Altman, *Berl. klin. Woch.*, 1908, xlv, 494.

mann[31] and cholesterin was found inactive by Levaditi and Yamanouchi.

My study of this topic was directed to the ascertainment whether qualitative or quantitative differences existed between extracts of normal and syphilitic blood and liver. The extraction was made with eight volumes of 95 per cent. alcohol at 42° C. for six days. The liver was made into a pasty mass by grinding and straining before extraction. The sera and coagula from syphilitic and normal bloods were extracted with ten volumes of 95 per cent. alcohol at 37 C. for five days. Before testing "the antigenic value" of the extract of each sample I removed salts by taking up the lipoids with ether. The ether soluble substances were then dried and fractionated with acetone.[32]

The following table gives the results:

			Acetone Insoluble.	Acetone Soluble.
Liver of a congenital syphilitic child.		15 grams	0.105 gram[33]	0.195 gram
Liver of a non-syphilitic child.		35 grams	0.665	0.250
Liver of a normal rabbit.		20 grams	0.540	0.140
Syphilitic coagulum.	I	20 grams	0.054	0.070
	II	17 grams	0·035	0.050
	III	15 grams	0.015	0.050
Normal coagulum.	I	32 grams	0.059	0.070
	II	20 grams	0.026	0.042
	III	28 grams	0.035	0.058
Syphilitic serum.	I	11.5 c.c.	Alcoholic extract without fractionation 0.205 gram.	
	II	11.5 c.c.	Alcoholic extract without fractionation 0.210 gram.	
Normal serum.	I	11.5 c.c.	Alcoholic extract without fractionation 0.202 gram.	

The lipoidal content of different samples of blood coagula varied rather irregularly, but this may be due to variable consistence of the coagula. In general it appears to be somewhat lower than with the sera, and many times less than with the livers.

[31] Fleischmann, *Berl. klin. Woch.*, 1908, xlv, 490.

[32] For redistilled acetone employed in the experiment I am obliged to Dr. P. A. Levene.

[33] The ratio between the acetone-insoluble and acetone-soluble portion in this sample of syphilitic liver is very different from the normal specimens, but I found it impossible, at the time, to secure a second syphilitic liver to confirm this result.

The ratio between the acetone-insoluble and acetone-soluble portion is quite the reverse in the liver and the blood coagula. In the liver the acetone-insoluble lipoids exceed the acetone-soluble lipoids, while the contrary is the case with the coagula.

Two cubic centimeters of the cerebro-spinal fluids derived from three cases of general paralysis and one non-paralytic patient yielded upon extraction 0.003 to 0.004 gram of lipoids each.

In determining the antigenic value of these extracts solutions of uniform concentration were made with the acetone-insoluble and acetone-soluble fraction of each coagulum or liver: thus 0.1 gram of the substance in question was dissolved in a few cubic centimeters of methyl alcohol and then emulsified in 100 c.c. of 0.9 per cent. salt solution. For the standard of antigenic value lecithin from two different sources (Lecithin "Agfa" Merck and one of my own preparations from beef blood corpuscles), were used. A syphilitic serum with the titer of 0.004 c.c. was employed as "antibody" in the amount of 0.1 c.c.

It was found that the antigenic strength of the acetone-soluble fraction of the extract of blood coagula, irrespective of their sources, was somewhat stronger than the acetone-insoluble fraction. With the extracts of livers the acetone-insoluble fraction was slightly more effective than the acetone-soluble fraction.

It was also found that *the lipoids extracted from syphilitic sera or blood coagula with alcohol can act as "antigens" for the "antibody" found in corresponding native sera.* There was no evidence that any qualitative or quantitative difference exists between the lipoids extracted from syphilitic and those extracted from normal liver, blood sera and blood coagula.

Just which lipoids are responsible for the powerful antigenic properties of the acetone-soluble portion has not been ascertained, but I can exclude cholesterin from the possible agents; for I tested negatively cholesterin preparations from sheep's brain, and gall stones and a sample prepared by Merck. Protagon, cholin and neurin did not exhibit in my hands convincing antigenic properties. I can confirm Levaditi in the finding that sodium glycocholate and sodium taurocholate can act as "antigens" but I found the

quantities required very large compared with lecithin and other antigenic lipoids. Sodium cholate acted in a similar way to the other two bile salts.

SUMMARY.

1. The high value in respect to complement-binding exhibited by blood sera from syphilitics and spinal fluids from general paralytics is associated with an excessively high content of globulin, but there does not exist a direct quantitative relation between the two. Cases of secondary syphilis which have been under prolonged and proper medication do not exhibit the globulin increase and usually fail to give the Wassermann reaction. The active substances entering into the Wassermann reaction are precipitable with the globulin and chiefly with the euglobulin fraction of the fluids.

2. Temperatures of 70° to 76° C. destroy the active substances. Exposed to sunlight the active substances deteriorate slowly. A photodynamic substance such as eosin, under the direct influence of the sun, brings about their complete and rapid destruction. This effect does not occur in the dark. The active substances are subject to tryptic and peptic digestion and are destroyed by weak acids and alkalies.

3. The active substances in the blood sera and spinal fluids cannot be separated from them or from the globulin precipitate by alcohol.

4. There are contained in the alcoholic extracts of normal and syphilitic blood and organs certain acetone-soluble lipoids which possess high antigenic values for the Wasserman reaction. Cholesterin is inactive and the bile salts less active than the lipoidal bodies.

5. Sodium cholate is about as active as sodium taurocholate and glycocholate, but neurin and cholin are inactive.

I wish to express my cordial thanks to Dr. Victor C. Pedersen (House of Relief), Dr. MacFarland (Sloane Maternity Hospital), Dr. Draper (Presbyterian Hospital), Dr. Sutton and Dr. Murphy (City Hospital), Dr. Lapowski (The Good Samaritan Hospital) and Dr. Mannheimer (The Lebanon Hospital) for the courtesies and assistances extended to me in securing the materials for this

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study. For the cerebro-spinal fluids I am under deep obligation to Dr. Moore of the Manhattan State Hospital in permitting me to work on the materials collected by him. To Dr. Wollstein I am indebted for the livers of syphilitic and normal children.

It gives me pleasure to acknowledge the invaluable assistance rendered me by Dr. R. V. Lamar during the execution of the present work.

PHAGOCYTIC IMMUNITY.[1]

By J. C. MEAKINS, M.D.

(From the Pathological Laboratory of the Presbyterian Hospital, New York.)

In 1895 Denys and Leclef (1) made the observation that the serum of animals immunized to streptococcus was necessary for the phagocytosis of virulent streptococci *in vitro* as well as in the body. They found that normal serum was powerless to produce ingestion of such streptococci by the leucocytes. By further experiments (2) they came to the conclusion that the serum acted upon the bacteria and not upon the leucocyte as previous workers had thought. Later Leishman (3) devised an ingenious method for the demonstration and estimation of phagocytosis. While working with this technique Wright (4) has demonstrated beyond doubt that the serum acts upon the bacteria preparing them for phagocytosis and furthermore has shown that normal serum contains a certain amount of the substance necessary for phagocytosis. To this body he has given the name " opsonin."

By a comparison of the number of organisms ingested by the leucocytes in the presence of serum to be tested with the number ingested in the presence of normal serum Wright has established what he has called the " Opsonic Index." By this method a quantitative difference between the normal and abnormal serum may be determined up to a certain point but the factor of error is so great and the difference of the end reaction so slight that the results of its use have been unsatisfactory. It is impossible by this method to demonstrate an opsonic power of serum comparable with that of the bactericidal power. If a blood serum were shown to have a bactericidal power of four times normal, the fact would have little importance; yet up to the present time, phagocytic activity four times normal has been seldom recorded by the opsonic index.

Phagocytic immunity may be many times the normal, but the pres-

[1] Received for publication October 26, 1908.

ent technique does not afford us a means of determining such increase. Neufeld and Hüne (5), closely followed by Klien (6), have independently shown that there does exist a true phagocytic immunity in animals which have been infected with non-lethal doses of certain living organisms, and they have devised a means whereby it may be demonstrated.

Dilution of the blood serum, so successfully employed in the quantitative estimation of the bactericidal and agglutinative powers, has been thought by few workers applicable to the estimation of the phagocytic substances. It was by this means that Neufeld and Hüne first demonstrated a power to promote phagocytosis quantitatively comparable with the other properties of immune serum.

Their technique is closely allied to Wright's but they have found that by progressive dilutions a point can be reached where the opsonins disappear. In the present work I have closely followed their procedure which I shall briefly outline.

The blood serum is collected in the usual manner both from the patient or animal infected and from a normal individual of the same species. The leucocytes are also collected as in Wright's work and a suitable emulsion of the infecting organism is prepared. The blood serum is then diluted to various strengths, which may reach one part in one thousand or higher if necessary. Then capillary pipettes are prepared as described by Lieschmann and equal parts of diluted serum, leucocytes and bacteria are mixed and incubated at 37° C. for a definite length of time, varying with the organism used. At the end of this period smears are made and suitably stained. In addition to the pipettes containing immune and normal serum one is prepared by substituting 0.85 per cent. salt solution for the serum and treated as described above. The average number of bacteria per leucocyte in the preparation with salt solution is taken as the degree of spontaneous phagocytosis. The preparation made with the most diluted serum which shows approximately this number is taken as the point where opsonins have ceased to be active. If for example the preparation with salt solution shows a phagocytic average of 0.5 bacteria per leucocyte and all the dilutions of serum up to 1 in 600 materially exceed this figure, then the phagocytic power is taken as 1 in 600, or as 600, which shall be the manner of

designation in the following experiments. The dilution of 1 in 600 is obtained from a dilution of 1 in 200 by mixing together equal parts of diluted serum, leucocytic emulsion and bacterial emulsion so that the serum is again diluted threefold, a final dilution of 1 in 600 being obtained.

The results obtained with the serum of a rabbit immunized to tubercle bacilli are cited for example:

Dilution.	Bacteria per Leucocyte with Serum of Normal Rabbit.	Bacteria per Leucocyte with Serum of Immune Rabbit.
1– 3	7	8
1– 30	.29	9
1– 150	.4	6
1– 300	.43	1.6
1– 600	.5	.35
1–1200	.2	.4

Salt solution—0.45 bacteria per leucocyte.

In this case the ordinary opsonic index would have been 1.1. With the method of dilution the opsonic power of the normal serum practically ceased below a dilution of 1 in 30, while the phagocytosis with immune rabbit's serum did not equal that with salt solution until a dilution of 1 in 300 at least was reached, that is more than ten times the normal. Occasionally instances are met with, where, in the undiluted state, the normal serum causes greater phagocytosis than the immune serum, yet the latter may show a much greater power when both are diluted, as the following protocol will demonstrate.

Dilution.	Bacteria per Leucocyte with Serum of Patient with Typhoid Fever on Tenth Day of Disease.	Bacteria per Leucocyte with Normal Serum.
1– 3	10	14
1– 30	6.8	.7
1–150	5.6	.5
1–300	.8	.9
1–600	.5	.6

Salt solution—0.6 bacteria per leucocyte.

Another point of interest, which has been frequently observed is the apparently increased opsonic power of the diluted over the un-

diluted immune serum. Such increase is seen in the following protocol:

Dilution.	Typhoid Bacilli per Leucocyte with Serum from Patient Recovering from Typhoid Fever.	Typhoid Bacilli per Leucocyte with Normal Serum.
1– 3	9	10.5
1– 30	9	.8
1– 150	14	.6
1– 300	10.5	.65
1– 600	4.3	
1– 900	.9	
1–1200	.6	
1–1500	.65	
1–1800	.7	

Salt solution—.7 bacteria per leucocyte.

In the greater part of the experimental work performed on animals for the production of opsonic immunity bacterial vaccines have been used, and the results have failed (7) to show the production of an immunity of a high degree (16). When, however, the infectious diseases in man have been closely imitated by the injection of living organisms in an attenuated form or in non-lethal, doses very striking results have been obtained. The injection of living *Bacillus typhosus* (6), *Bacillus paratyphosus* and the vibrio of cholera (5) has produced marked opsonic immunity.

In the following experiments living organisms have been used, and in all instances save with *Bacillus tuberculosis* the injections have been subcutaneous, since this method of inoculation seems to produce a better reaction than intraperitoneal or intravenous inoculation. Realizing that the different groups of bacteria may produce varied forms of immunity I have selected members of the different classes of bacteria most common in the causation of infectious diseases in the human subject.

Acute infections.

I. Bacilli (producing bactericidal immunity).

(*a*) *B. dysenteriæ* of Flexner.

(*b*) *B. dysenteriæ* of Hiss.

II. Pyogenic Cocci.

(*a*) *Staphylococcus aureus.*

(*b*) *Streptococcus pyogenes.*

Chronic infection.

Bacillus tuberculosis.

BACILLUS DYSENTERIÆ.

EXPERIMENT I. On June 25, 1907, one loopful of *Bacillus dysenteriæ*, Manila, type **Y**, was injected subcutaneously into a rabbit. This dose was repeated at intervals of from five to sixteen days and gradually increased until on August 29, two thirds of an agar slant was injected. During this period the opsonic and agglutinative power of the rabbit's serum were estimated and controlled by normal serum and salt solution. The microscopic technique was employed for the

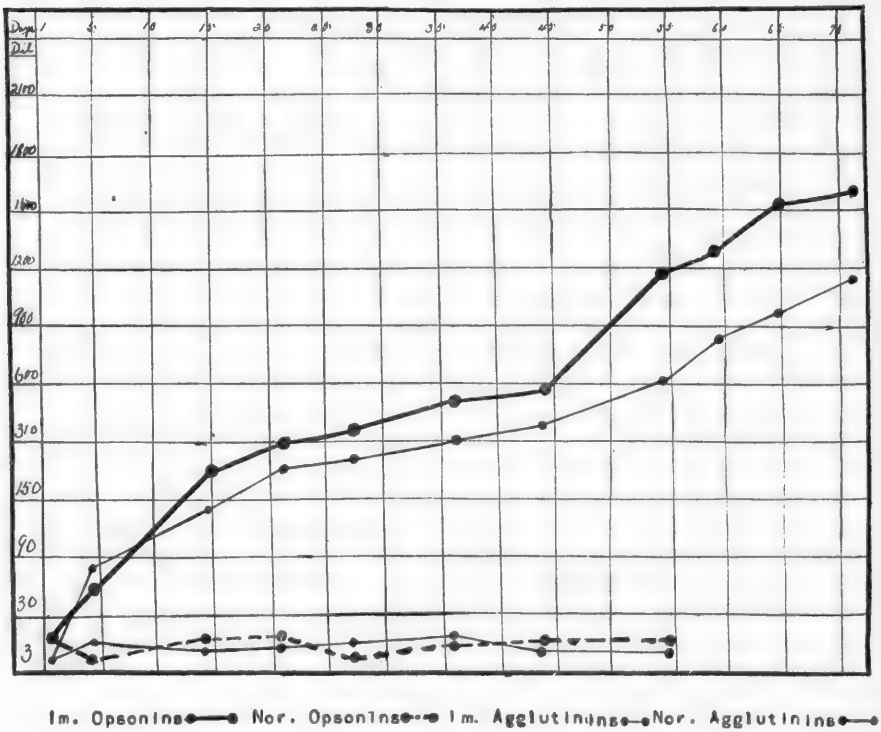


CHART I. *Bacillus dysenteriæ, Manila, type* **Y**.

estimation of the agglutinins and the length of time before the result was taken was one hour. In the estimation of the opsonic power the preparations were incubated for eight minutes as a longer time produced marked degeneration of the intracellular organisms. The slides were stained with Hasting's modification of Jenner's stain and fifty leucocytes counted in each. This experiment is demonstrated by Chart I.

The highest opsonic power in the normal serum was 10, while the opsonins in the immune serum rose to 1600. The highest agglutinative power of the normal was 10 and of the immune serum was 1100.

EXPERIMENT II. In this case *Bacillus dysenteriæ*, Manila, type (Flexner), was used for immunization and doses similar in size and frequency to those of the preceding experiment were employed. The animal was under observation from June 26 to September 5, 1907, or seventy-two days.

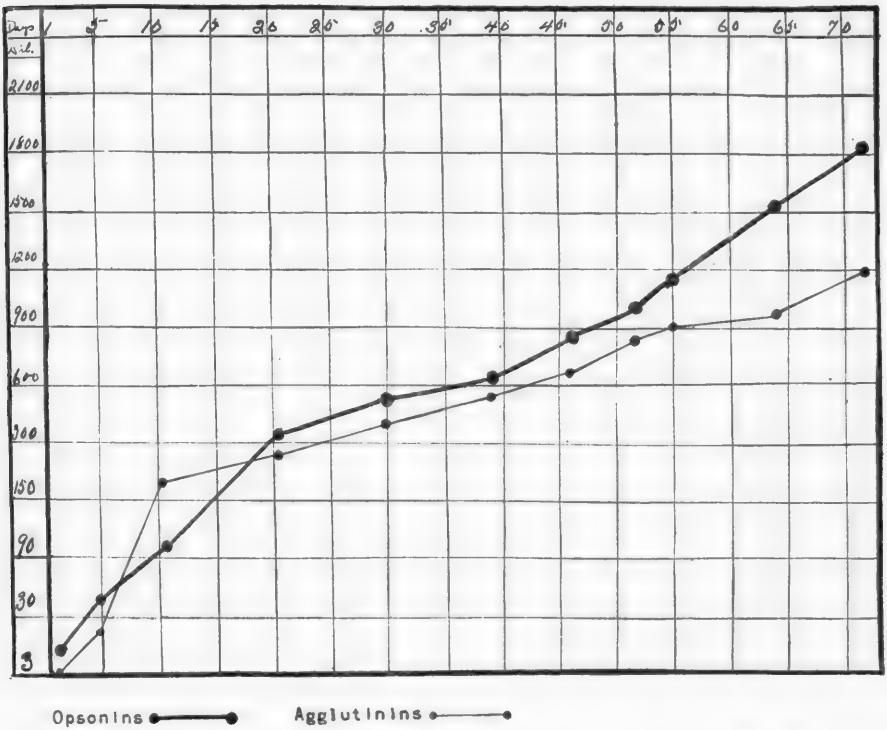


CHART II. *Bacillus dysenteriæ*, Manila, type (Flexner). The curves indicating normal opsonins and agglutinins are identical with those of Chart I.

The result of the last experiment (Chart II) was very similar to that of Experiment I. The highest opsonic power was 1900 and the agglutination reached 1200 while the normal agglutinins never exceeded 10.

In examining the results of these two experiments the production of phagocytic immunity to the dysentery bacillus is evident, and the close parallelism between the opsonic and agglutinative curves suggests that there may be a relationship between these two bodies.

EXPERIMENT III. A rabbit was immunized to *Bacillus dysenteriæ* (Flexner) as in Experiment II but was under observations for a much longer period, namely 144 days. In this experiment besides the opsonic and the agglutinative power, the bactericidal power was estimated. The technique for the estimation of the bactericidal power was that of Neisser (8) as modified by Stern and Körte (9) and later by Klien. The serum of a normal rabbit was frequently examined to determine for comparison its content of immune bodies. The following table shows the maximal dilutions in which positive results were obtained.

Agglutinative power	1–10?
Phagocytic power	1–10
Bactericidal power	nil.

For the first 98 days the opsonic, agglutinative and bactericidal powers all gradually increased as shown in Chart III. But at this period the animal developed an abscess of the abdominal wall at the site of inoculation; from the pus *Staphylococcus aureus* was cultivated. It was not found necessary to incise this abscess which disappeared spontaneously. For a period of 16 days (March 14 to 30) on account of unavoidable circumstances I was unable to inject the animal, and during this period it lost a little weight and did not seem as well as usual. Its general condition gradually grew worse after this date and it died on May 5, 1908. On March 18, 1908, the phagocytic power was 1,300, the bactericidal power 950, while the agglutinative power was slightly lower. After this date and synchronous with the illness of the animal the opsonic and bactericidal power quickly dropped towards normal. Two days before death the former was 60 and the latter 30. On the other hand, the agglutinins progressively increased and just before death reached their highest point, namely 950.

The autopsy was performed one hour post mortem. The animal was much emaciated, and the viscera showed only cloudy swelling. There was no abscess formation and the intestines appeared normal. Cultures from the heart blood, liver, spleen, lungs and kidneys gave *Bacillus dysenteriæ* in pure culture.

An analysis of Chart III gives a very good idea of the immunity produced in animals by the inoculation with living cultures of *Bacillus dysenteriæ* and allied organisms. An absolute comparison between phagocytic and bactericidal power by the technique used, is obviously impossible. The parallelism between the opsonic and bactericidal curves is evident and affords some evidence that the opsonins are related to the amboceptors, but later experiments will demonstrate the fallacy of this assumption.

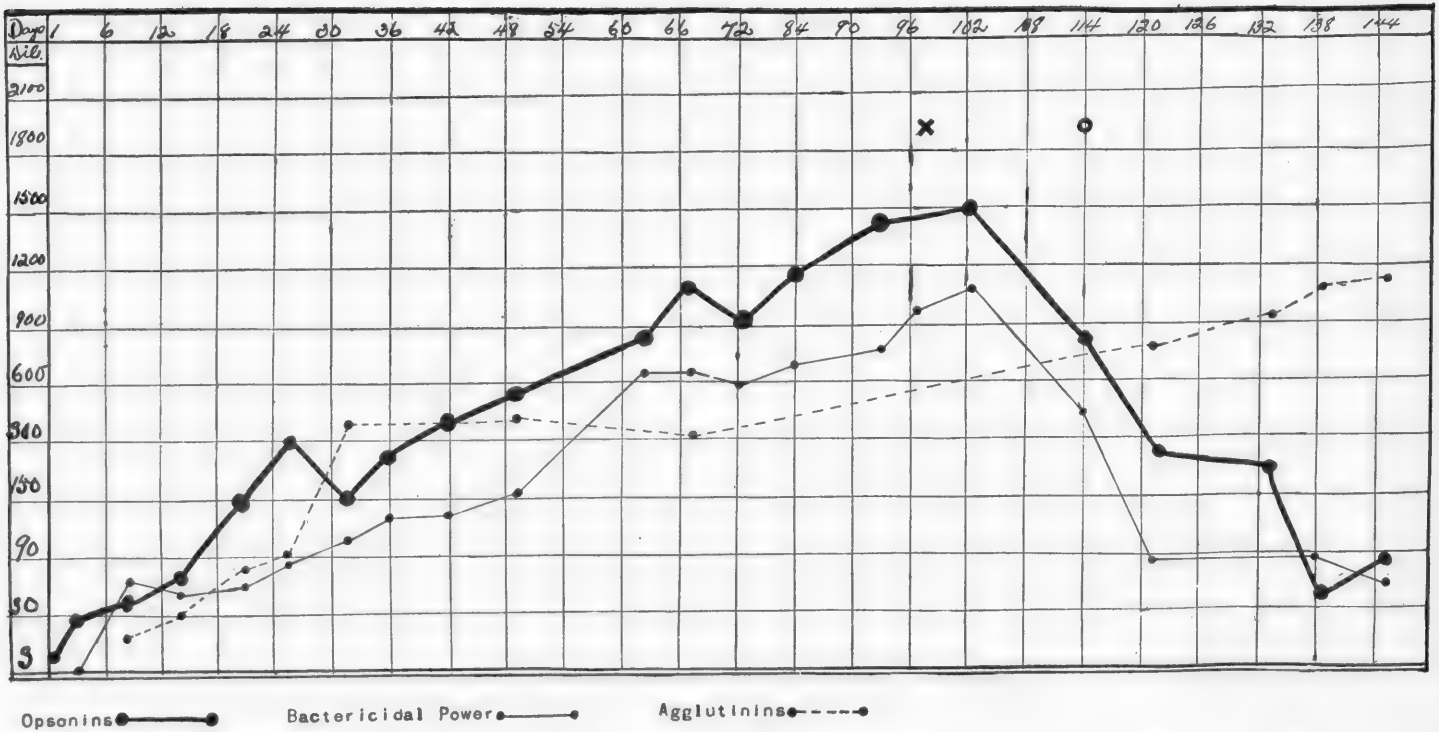


CHART III. *Bacillus dysenteriæ* (Flexner). Curves indicating normal opsonins and agglutinins are identical with those of Chart I.
X = March 14. O = March 30.

The parallelism of the curves of the agglutinins and opsonins observed in Experiments I and II was again found up to a certain point. Nevertheless the great divergence of the curves after March 30, is against the view that these bodies which are in common specific and thermostabile, are related or identical. Experiments with other organisms will still further demonstrate that the agglutinins and opsonins do not always run parallel courses.

In the low dilutions of the highly immune sera the agglutinins may have produced a favorable condition for phagocytosis, since agglutination occurs quickly and the leucocytes can readily find clumps of opsonized bacteria. But in spite of this fact when the agglutinins were still increasing, the opsonic curve steadily decreased. On the other hand, with the high dilutions, for example from 700 to 1300, the agglutinins exerted no power in one hour, whereas the opsonins were very active in eight minutes.

The cause of the animal's loss of weight and illness after March 30, 1908, is hard to explain by facts admitting substantial proof. It seems probable that just prior to this date the animal was exerting all its powers of immunity to withstand the infection repeatedly administered, but the demand for immune bodies became greater than the supply and a drop of the opsonins and bactericidal power occurred between March 18 and 30. The inoculation on this date accentuated this depression which became more marked with each subsequent injection.

The great importance of the phagocytic and bactericidal substances in the protection of the body against infections is very well demonstrated in this experiment. Even when these bodies are being produced in large amounts by the animal organism, continued increase in the amount of infection may overwhelm the supply and lead to rapid consumption of the immune bodies which have lost mastery over the infection; actual diminution in their production may result. An example of an analogous condition is relapse in typhoid fever occurring in the presence of a highly bactericidal serum. Diversion of complement could not have been the cause of the progressive decrease in the bactericidal power noted in Experiment III as normal rabbit's serum was always added to the mixture which was tested, and on several occasions the usual amount was

doubled in order with certainty to satisfy with complement all the amboceptors which might be present.

Agglutinin, without the presence or aid of the other bodies of humoral immunity, appears to have little importance as a protective agent in infections due to *Bacillus dysenteriæ*. The phenomenon of Eisenberg and Volk (10), which gives evidence of the presence of agglutinoids, or proagglutinoids according to Shiga (11), was observed in these experiments in the low dilutions. The continued increase of the agglutinins until death has shown that prognosis may be unfavorable even though they are abundant and accords with the frequent observation of relapses or recurrences in typhoid fever although the serum shows high agglutination.

The increase of the bactericidal power and aggutinins in these experiments coincide with similar increase found by Shiga in horses inoculated with the *Bacillus dysenteriæ*.

The conclusions, which may be drawn from this series of experiments are as follows:

I. A high opsonic power may be developed against the bacillus of dysentery.

II. The bactericidal and opsonic power of the serum may decrease when the agglutinative power is increasing.

III. A close parallelism has been observed between the quantitative changes of the opsonins and amboceptors occurring with immunization to *Bacillus dysenteriæ*.

STAPHYLOCOCCUS AUREUS.

EXPERIMENT IV. On December 17, 1907, one-half an agar slant of *Staphylococcus aureus*, obtained from a case of cellulitis, was heated to 65° C. for one-half hour and injected into a rabbit. This injection was followed in seven days by all of the growth from an agar slant, similarly heated. These inoculations were given with the hope of producing some immunity before living organisms were inoculated. The result in this animal was very satisfactory, and subsequent inoculations produced no local suppuration. The injections of living organisms were given at intervals of from four to six days, beginning with one loopful and increasing the dose slightly with each inoculation. Unfortunately the animal was pregnant and abortion was produced; and a severe mixed infection of the genito-urinary tract followed. The animal was killed after it had been under observation for a period of forty-four days. The autopsy showed no other lesion but that mentioned above. The kidneys contained large abscesses and the uterus formed a bag of pus. Cultures were taken from the bladder, uterus and

kidneys, and in all instances there was a growth of *Staphylococcus albus,* streptococcus and a bacillus giving the reactions of *Bacillus coli.*

The opsonins and agglutinins were estimated during the time the animal was under observation (Chart IV). Controls made frequently with the serum of a normal rabbit have shown that these bodies are normally present but never active in dilution above 1 in 10 and sometimes they are not active even in this dilution. The technique was similar to that employed in the first set of experiments with the exception that the preparations were each incubated for fifteen minutes instead of eight. The serum of the inoculated animal revealed a marked increase in the amount of opsonins. At

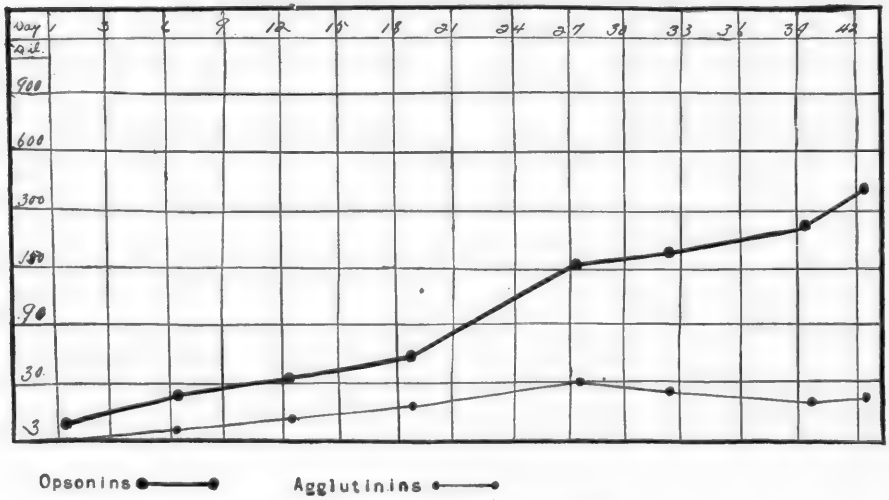


CHART IV. *Staphylococcus aureus.*

first the reaction was slow but later progressed more rapidly and at the time of death opsonic activity was 400, or forty times the normal. The agglutinins, however, never rose above 30 and this point was only reached on one occasion.

In no experiment was any intra- or extracellular lysis of the organisms noticed, and at all times the cocci stained very well and no shadows were seen.

Further experiments with staphylococci were unsuccessful as the

animals developed abscesses at the points of inoculation and frequently pyæmia resulted. Nevertheless from the one observation just recorded it appears that phagocytic immunity of a fairly high degree can be produced against *Straphylococcus aureus,* and further evidence will show that phagocytic immunity plays an important rôle in the destruction of the pyogenic cocci.

STREPTOCOCCUS PYOGENES.

The strain of streptococcus used in this experiment was obtained in blood culture from a fatal case of malignant endocarditis and pyaemia of short duration. It was very virulent to mice, killing them in small doses in twenty-four hours.

EXPERIMENT V. On May 24, 1908, the animal was injected with one-half the growth from an agar-slant culture of streptococci. The dose was irregularly increased, inoculations being given at intervals of from five to nine days. The animal was under observation for a period of ninety-seven days and during this time the opsonic, bactericidal and agglutinative power of the blood serum were frequently estimated (Chart V). The technique was similar to that described for *Staphylococcus aureus* but it was found of great advantage in the plating for the bactericidal experiments to use blood agar plates. These were prepared by adding 1 c.c. of sterile defibrinated beef blood to 10 c.c. of melted agar at 42° C. Control plates were always made to be sure that the culture media were sterile. In this medium the colonies of streptococci could be readily counted as they were surrounded by a small zone of hæmolysis.

Experiments were made with normal serum and the following results were almost constantly obtained:

Bactericidal power	0
Opsonic power	10
Agglutinative power	0

The results of these experiments are graphically demonstrated in Chart V.

The highest point reached by the bactericidal power was 100 and this level was reached only on one occasion. In fact the bactericidal power seldom rose above 30 and the power of agglutination was slightly higher. The end reaction for both of these bodies was very unsatisfactory, except in very low dilutions. In fact, when the experiments were carried out in duplicate, the results were often very indefinite.

The estimation of opsonins, on the contrary, had a definite result;

opsonins steadily increased in quantity until an opsonic activity of 3,000 was obtained. The extracellular cocci showed no degeneration even when the serum had the greatest power. On the other hand, when the animal had developed an opsonic power of 600,

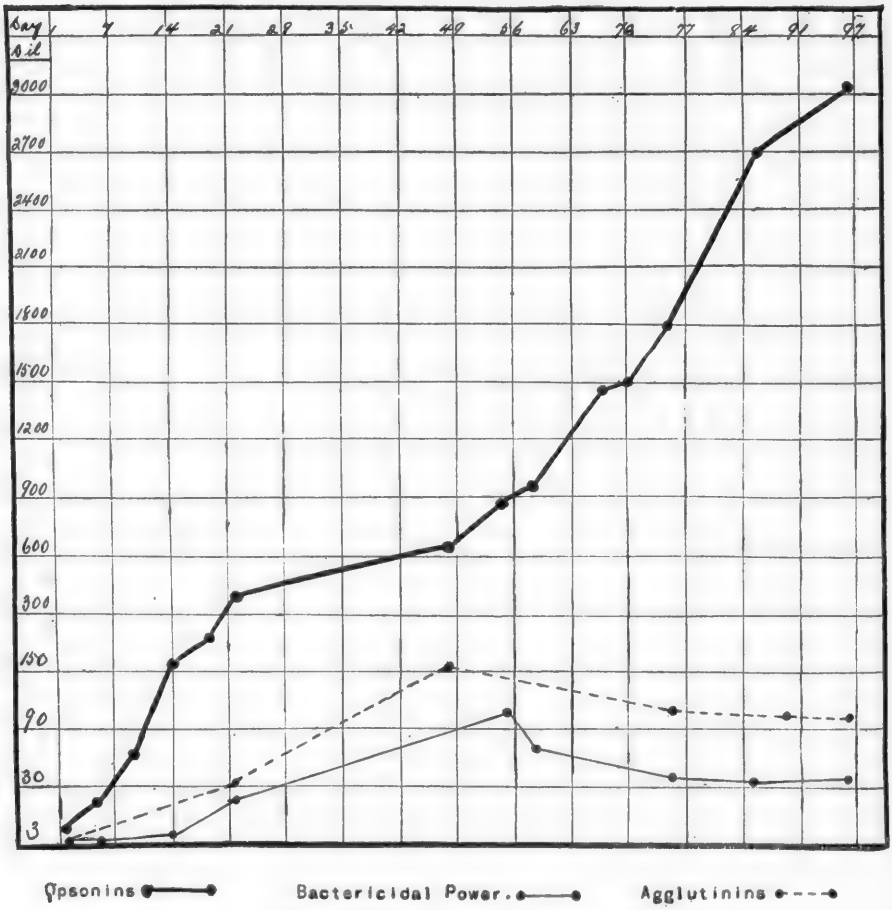


CHART V. *Streptococcus pyogenes.*

degeneration of the intracellular organisms was first observed. This degeneration became more marked as the opsonic power in-

creased, but at no time did bacteria exhibit the degree of degeneration found in the experiments with *Bacillus dysenteriæ*.

The high degree of opsonic immunity developed in this animal is strong evidence that phagocytosis is the most important factor in the destruction of streptococci. It confirms the observations of Neufeld and Rimpau (12) who have found that phagocytosis is very active in the destruction of streptococci *in vivo* whereas bactericidal power although increased plays a minor part. Ruediger (13), however, when working with the serum of patients suffering from erysipelas, found that the opsonic power was but slightly increased above normal and the bactericidal power was absent. He gives details of but one observation of the phagocytic power and the method of estimation is somewhat similar to that employed in the above experiments. Although the bactericidal power may not be greatly increased in infection with streptococcus, I agree with Neufeld and Rimpau that it is present: bactericidal substances are probably present in small amount but the opsonic power plays the greater rôle.

Neufeld (14) has demonstrated that agglutinins are produced in animals immune to streptococci, although seldom present in a dilution over 1 in 100. On one occasion he reports their presence in a dilution as high as 1 in 500. In the present experiments the highest point reached has been a dilution of 150; this fact corroborates the previous observations. The lack of parallelism between the curves of the opsonins and amboceptors in this experiment is a strong argument against their identity. An attempt to explain the absence of bactericidal power by deviation of complement would be useless as abundant complement has been added in each experiment and bactericidal bodies at no time gave any indication of material increase.

There is likewise an entire lack of parallelism between the opsonins and the agglutinins in the immunity produced in rabbits to *Staphylococcus aureus* and the *Streptococcus pyogenes*.

The conclusions that may be drawn from these experiments are as follows:—

I. A high degree of opsonic immunity to *Streptococcus pyogenes* and *Staphylococcus aureus* may be produced in rabbits.

II. Opsonins are more important than the other known immune

bodies in causing protection against the pyogenic cocci: in rabbits immunized to *Streptococcus pyogenes* bactericidal substances and agglutinins are produced in small amounts and apparently play a minor rôle in the active immunity against this organism.

III. Opsonins are not identical with the amboceptors of the bactericidal substances.

BACILLUS TUBERCULOSIS.

For investigation of phagocytic immunity in experimental tuberculosis the selection of the strain of bacilli to be employed is of great importance. The organism must not be of high virulence. Through the Rockefeller Institute for Medical Research I obtained a strain of tubercle bacilli, attenuated by Dr. Trudeau, which even in large doses was not fatal to rabbits and the animals recovered although they showed for a short time signs of infection. The animal was subsequently inoculated with a second strain of greater virulence in order that the immunity produced by the attenuated culture might be tested.

The emulsion of tubercle bacilli employed for the estimation of the opsonic power was killed by exposure to direct sunlight for twenty-four hours and then ground up in a small test-tube with a glass rod under aseptic precautions as described by Sellards and Jeans (15). In this manner a very satisfactory emulsion was obtained; it was free from clumps and fragmentation of the bacilli was very slight. The preparations were incubated for fifteen minutes; the smears were stained in carbol-fuchsin at 37° C. for twelve hours, decolorized in 95 per cent. alcohol for ten minutes and counterstained with Loeffler's methylene blue for five minutes. This method stained the leucocytes very distinctly and gave them a clear outline; there was little of the destruction which occurs when greater heat or when acids are employed. One hundred leucocytes were counted in each slide instead of fifty as in the preceding experiments.

EXPERIMENT VI. On August 9, 1908, a rabbit received into the ear vein 2 c.c. of an emulsion of non-virulent tubercle bacilli. The emulsion was opaque and was freed from clumps by grinding the bacilli very carefully and then centrifugizing the emulsion until the supernatant fluid was cloudy but contained no

visible particles. The opsonic power was estimated as described above and controlled with normal serum and salt solution (Chart VI). Normal sera always showed an absence of phagocytosis at 30, in fact its strength may have been much less, as dilutions between 3 and 30 were not made.

In the treated rabbit the opsonic power decreased below normal for a week after inoculation but suddenly began to increase and this

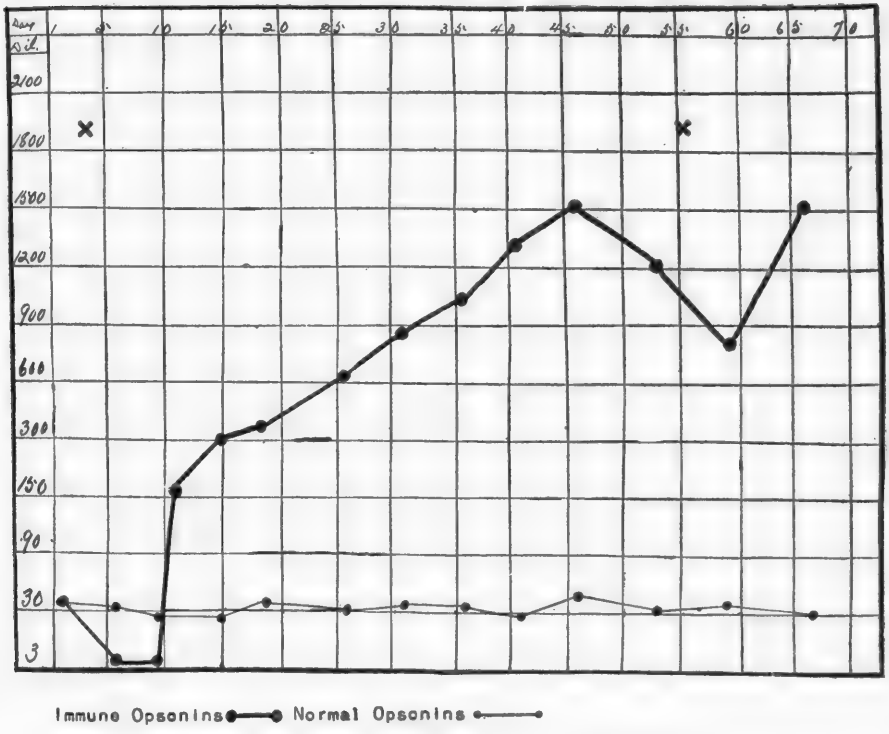


CHART VI. *Bacillus tuberculosis.* X, X = inoculation.

increase steadily progressed until a power of 1500 was reached forty-four days after the original inoculation. There was then a slight drop and the animal was again inoculated on October 3, 1908, fifty-three days after the first injection. On this occasion an emulsion of the virulent culture was prepared by the method described above and the intravenous method was used for inoculation. The

opsonic power continued to fall slightly but eleven days after the second inoculation it had regained its high point of 1500.

During the period of observation the animal was frequently weighed and the curve of weight closely followed the opsonic curve; at first there was a sudden loss of weight followed by a slow recovery until the original weight was exceeded. After the second inoculation there was no drop in weight coresponding to that following the first injection.

As no satisfactory method has yet been devised whereby the bactericidal power against the tubercle bacilli can be estimated a comparison between this body and the opsonins is imposible. In none of the experiments was any lytic action on the extracellular organisms seen, though occasionally in the low dilutions of the most powerful sera an increased beading of the bacilli seemed to have occurred, but the change was too indistinct to justify a definite opinion concerning the lytic action of the serum.

Observations made by several workers indicate that agglutinins occur with tuberculous infections but they are not found in large amounts.

The drop in the phagocytic power after each inoculation was most likely due to the demand for a greater quantity of protective bodies than the body was capable of providing so that the amount present in the serum was temporarily reduced; but the animal was in such good condition that the loss was soon replaced. As the inoculation was not repeated the increased demand did not continue whereas in Experiment III inoculations were repeated and the animal finally died. That this drop in the opsonins may have been the " negative phase " described by Wright is probable.

The animal used in this experiment is rapidly gaining weight, twenty-eight days after it received an inoculation with virulent tubercle bacilli sufficient to cause death if the animal had not developed immunity from the first injection.

It has been stated above that there is at present no evidence to show the development of bactericidal immunity in tuberculosis. Observations of several workers indicate that development of agglutinins in tuberculous infection is limited. It is, therefore, reasonable to suppose from this experiment that opsonins play an important part in tuberculous immunity.

The following conclusions may be drawn from the experiment just described.

I. Animals immunized with living tubercle bacilli may develop phagocytic immunity of a high degree.

II. Such immunity may help to protect the animal from a lethal dose of virulent tubercle bacilli.

With slight modification the method used in these experiments is applicable for routine clinical observations, and might as suggested by Klien be used as a means of diagnosis. The further application of this method of observation may add greatly to the knowledge of the phenomenon of phagocytosis especially in those infectious diseases of man, in which phagocytic immunity is known to play a more or less important part.

In conclusion I wish to express my sincere thanks to Dr. Opie for his valuable aid and advice in this work.

BIBLIOGRAPHY.

1. Denys and Leclef, *La Cellule,* xi, 175, 1895; Denys and Marchand, *Bulletin de l'Acad. r. de Belgique,* 1896, x, 249.
2. Denys, *Centralbl. f. Bakt., Abt. I,* 1898, xxiv, 685.
3. Leishman, *British Med. Jour.,* 1902, i, 73.
4. Wright, *Proc. Royal Soc.,* 1904, lxiii, 128.
5. Neufeld and Hüne, *Arb. a. d. k. Gesundheitsamte,* 1907, xxv, 164.
6. Klien, *Johns Hopkins Hospital Bulletin,* 1907, xviii, 245.
7. Moss, *Johns Hopkins Hospital Bulletin,* 1907, xviii, 237.
8. Neisser, *Münch. med. Woch.,* 1901, xlviii, 697.
9. Stern and Körte, *Berl. klin. Woch.,* 1904, xli, 213.
10. Eisenberg and Volk, *Zeitsch. f. Hygiene,* 1902, xl, 155.
11. Shiga, *Zeitsch. f. Hygiene,* 1902, xli, 355.
12. Neufeld and Rimpau, *Deut. med. Woch.,* 1904, xl, 1458.
13. Ruediger, *Jour. of Infectious Diseases,* 1906, iii, 156.
14. Neufeld, *Zeitsch. f. Hygiene,* 1903, xliv, 161.
15. Sellards and Jeans, *Johns Hopkins Hospital Bulletin,* 1907, xviii, 232.
16. Simonds and Clarke, *Jour. of Infectious Diseases,* 1908, v, 1.

ON THE RELATION OF TETANY TO THE PARATHYROID GLANDS AND TO CALCIUM METABOLISM.[1]

By W. G. MacCALLUM,

Associate Professor of Pathology,

AND CARL VOEGTLIN,

Assistant in Medicine in Charge of the Chemical Laboratory of the Medical Clinic, Johns Hopkins University, Baltimore.

(From the Hunterian Laboratory and the Medical Clinic.)

Notwithstanding the fact that a great deal of study has been devoted to tetany and tetanoid conditions and to the nature of the parathyroid glands in their relation to these conditions in recent years the subject is still far from being perfectly understood. We have not yet a sufficiently large number of carefully investigated cases of some of the forms of tetany to speak with full certainty of the clinical phenomena and underlying pathological conditions, but this material is rapidly accumulating and rapidly becoming more valuable as the new light shed upon the subject by experimental work makes it possible to study the cases more intelligently.

v. Frankl-Hochwart in his notable monograph upon Tetany in Nothnagel's System subdivides the cases into a number of groups.

1. Tetany following or accompanying infectious diseases such as typhoid fever, cholera, etc.

2. Tetany in cases of gastric dilatation with stagnation of the stomach contents.

3. Tetany in infants and children thought by recent writers to be connected with nutritive disturbances and lesions of the parathyroids.

4. Tetany of so-called idiopathic nature occurring in workmen who ply certain trades such as those of the shoemaker, carpenter, etc.

5. Tetany associated with such diseases as osteomalacia, rickets, etc., possibly due as Erdheim suggests to lesions of the parathyroid gland.

[1] Received for publication November 2, 1908.

6. Tetany occurring in the course of pregnancy and lactation.

7. Tetany resulting from operative extirpation of the thyroid gland in which the parathyroids are also removed or destroyed.

In all these forms of the disease the symptoms are in their general character the same. They depend on increased excitability of the central nervous system which may be recognized by quantitative tests with faradic and galvanic currents or by the spasmodic rigidity of the muscles, frequently with violent twitchings which may even become so intense and general as to constitute an epileptiform convulsion. This is seen frequently in children, and sometimes, in dogs; such convulsions may closely resemble those of an epileptic. Fibrillary tremors of the tongue and distorting contractions of the facial muscles are often present and the jaws may be so tightly clenched that speech or the taking of food is impossible. Changes in temperature have not been well studied, but numerous instances are on record in which distinct elevation of the body temperature was observed. Our own attention has not until recently been especially directed to this point, but our casual observations have shown during the actual tetanic spasms in dogs rectal temperatures ranging from 38° C. to 43.2° C. In a recent instance in which the temperature of the dog, until the appearance of tetany, stood at the normal figure 39° C. it was found to register during the tetany 43.2° C. The administration of calcium in the way to be described, caused the muscular contractions to disappear and within half an hour the temperature sank to normal limits. The idea suggests itself that here as in cases of hyperpyrexia in tetanus, status epilepticus, intense muscular exertion as in a footrace, etc., the elevation of the temperature may be due merely to the fact that with violent muscular activity and excessive heat production, the heat regulating mechanism is for a short time inadequate and the elimination of heat is not rapid enough to compensate for the sudden heat production. Exact studies of the temperature in such animals will be published elsewhere. The pulse rate is generally quickened, but is sometimes not very greatly changed. Little mention is made in the literature of periods of tachypnoea, but in dogs this is one of the most striking features. It is doubtless an effort toward the regulation of the body temperature. But the condition has been so extensively described that it is

quite unnecessary to repeat the descriptions here since our results are concerned rather with the experimental study of the disease than with its clinical characters. It is indeed through experimental study that all of the information has been obtained which can lead to any therapy which may be of benefit in such cases and in view of the agitations designed to interfere with such studies, in this country it seems well to call attention to these results.

The fact appears to be thoroughly well established in spite of the dissenting voices of a few English investigators that the parathyroid glands are organs of independent nature and not developed from the thyroid or convertible into thyroid tissue. The evidence against this is of so insignificant a character as compared with the overwhelming evidence in its favor, that space need not be occupied here in discussing it. It may be said, however, that the argument for the unity of these two glands based on the chemical analyses of Gley, who found large amounts of iodine in the parathyroids has been disproven in the recent papers of Estes and Cecil[2] in which it was shown that the iodine content of the parathyroid glands is insignificant.

Nor is it less certain that the parathyroid glands exercise a specific function although we are as yet not perfectly informed as to that function. At least, we know well that the destruction of all the parathyroid glands results in the death of the animal with symptoms of tetany which is not in the least dependent, as was once thought, upon the destruction of the thyroid gland.

The work of Gley, Vassale, Generali, Horsely, Kocher, Erdheim, Pineles and many others sufficiently proves this, and in previous papers by one of us,[3] numerous experiments have confirmed these results and have convinced us beyond doubt or question as to the correctness of the observations. Most of our experiments have been performed upon dogs but Gley and others have extirpated the gland with similar results in rabbits, Horsley and Edmunds in monkeys, Erdheim in rats and Hagenbach in cats.

The objection has been raised that it is impossible to produce tetany in herbivorous animals, possibly on account of the difference

[2] Estes and Cecil, *Johns Hopkins Hospital Bulletin*, 1907, xviii, 331.

[3] MacCallum, *Medical News*, 1903, lxxxiii, 820; *ibid.*, 1905, lxxxvi, 625; *Centralbl. f. allg. Path.*, 1905, xvi, 385.

in the character of their food but we have shown in a recent paper[4] that if the extirpation be really complete it is possible to produce the most characteristic tetany in such animals as goats. It seems that the failures in these experiments, some of which occurred in our own hands, are probably due to the wide distribution of the parathyroid tissue which makes it difficult, if not impossible for the operator to recognize every focus of such tissue. Getzowa[5] and Michaud have recently pointed out the fact that such small masses of parathyroid tissue may occur deeply embedded in the tissue of the thyroid in human beings and we have repeatedly found this to be the case in dogs. There are, for example, several instances in which after the removal of four parathyroids, no tetany resulted, a phenomenon easily explained by the subsequent discovery of an extra mass of parathyroid tissue embedded in the thyroid or elsewhere. The extirpation of this extra nodule will bring about the development of tetany but it is surprising to note the extremely small size of the mass of parathyroid requisite to keep the animal from having tetany.

From this it is evident that the number of parathyroid glands is not by any means so constant as has generally been thought. Erdheim[6] points out that in rats their distribution is extremely variable and only the most careful study of several sections through all the organs of the neck is sufficient to give a trustworthy idea of their number. The inconstancy of the distribution of thymus and thyroid tissue is sufficient to make it probable that aberrant parathyroids might well occur also. Studies of the number and distribution of the parathyroid glands from a surgical point of view have been published by one of us[7] and by many other writers in this country and seem to show that the number and position depart from the typical form in very many cases. So, too, in dogs we have recently found cases in which six or eight distinct parathyroid glands were found in relation with the thyroid. Seldom, or never, however, have we succeeded in producing tetany by the extirpation of the parathyroid

[4] MacCallum, Thomson and Murphy, *Johns Hopkins Hospital Bulletin*, 1907, xviii, 333.

[5] Getzowa, *Virchow's Archiv*, 1907, clxxxviii, 181.

[6] Erdheim, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1906, xvi, 632.

[7] MacCallum, *Brit. Med. Jour.*, 1906, ii, 1282.

glands when we could find only three, and we have usually concluded that others were embedded in the thyroid as was proven by the subsequent extirpation of the thyroid and the production of tetany.

Nevertheless, in any long series of experiments there are some puzzling cases in which even though apparently every possible tissue which could contain parathyroid tissue has been extirpated the dog does not develop a frank tetany but may remain quite well or sink into a sort of apathy in which he is prone to infection and in which he finally dies. The exact nature of this is not clear but several things may possibly bear upon it. There may be a remnant of parathyroid tissue left too small to maintain perfect health, or the occurrence of a secondary infection, or the nature of their food may be sufficient to alter the character of the symptoms from those of outspoken tetany. We have referred at length to this matter in a previous paper[8] without reaching a conclusion and even now only those cases are clear in which at autopsy or on second operation remnants of parathyroid tissue are found embedded in the thyroid. Outspoken tetany is, however, not necessarily perfectly continuous even in those cases in which the extirpation of the parathyroids has been complete—there are intermissions in which the animal, while very sick and depressed does not show spontaneous twitching although the electrical excitability of the nerves may be increased. Others may live for a long time upon the verge of actual tetany, occasionally suffering from acute attacks but fairly well in the meanwhile. Beside this one may ameliorate the tetany for a time by bleeding and infusing salt solution so that although not permanently cured the animal is relieved from the acute symptoms for a time. On the whole it seems that the occurrence of these acute symptoms depends first of all, upon the complete removal of the parathyroid gland and then upon the degree of metabolic disturbance which results and which may perhaps be modified by the diet to a slight extent. In some instances in which dogs were kept fasting for the purpose of studying the changes in metabolism resulting from parathyroidectomy, we have been tempted to wonder whether their failure to develop tetany was due to the lack of food. This seems to be contradicted, however, by the fact that in several such cases

[8] MacCallum and Davidson, *Medical News*, 1905, lxxxvi, 625.

we have subsequently found minute masses of parathyroid embedded in the thyroids, and further by the fact that the tetany has often developed during such fasting.

It is of course possible that some of these animals, in which tetany was not observed had really passed through an attack with convulsions and tachypnœa at some time during the night when they could not be watched—indeed in some instances there were signs about the cage and in the position and general condition of the animal which would indicate this.

Although we have watched animals in which tetany had developed, for long periods and have observed as described above that they may be free from tetany except for attacks at long intervals, we have seldom seen a complete recovery after tetany had definitely appeared. It is interesting to read that v. Frankl-Hochwart[9] in his study of the ultimate fate of persons who have had tetany finds that they generally continue to show symptoms throughout their lives, but this chronic tetanoid condition can surely not refer to those cases in which transient attacks of tetany follow infectious diseases, pregnancy, lactation and nutritive disturbances.

In the previous papers referred to it was pointed out that the symptoms of tetany could be made to disappear by the subcutaneous or intravenous injection of an extract of the parathyroid gland—in other words, by an artificial replacement of the parathyroid. Since that time a number of investigators have published observations which confirm this fact and some have even proceeded farther and have found (Beebe) that the active principle of the gland is contained in the nucleo-proteid which is capable of being separated from the remaining albuminous substances by precipitation with acetic acid. The remaining albuminous substance is inert, but the solution of the nucleo-proteid is quite active in dispelling the symptoms of tetany. We have repeated this and can confirm Dr. Beebe's results to this extent. Doses of parathyroid material given by mouth have had no good effect in our hands although Dr. Halsted reports beneficial effects in a case with tetanoid symptoms which he has observed. That the subcutaneous administration of the extract of the gland has a remarkable effect was seen not long ago in a case which will doubtless be reported later by Dr. Branham.

[9] v. Frankl-Hochwart, *Wien. med. Woch.*, 1906, lvi, 309.

This was the case of a girl from whom a goitre had been extirpated together with portions, at least, of the parathyroid. The insufficiency thus produced resulted after seventy-two hours in a most violent attack of tetany in which the girl became very rigid and perfectly helpless. The fingers were contracted into the characteristic position, the feet extended and the jaws so tightly set that nothing could be forced into her mouth. An emulsion of four or five parathyroid glands of the cow was prepared aseptically and injected beneath the breast. Several hours later there was a complete relaxation of the rigid muscles and the patient fell asleep to wake up next morning feeling quite well. Four days later there occurred another similar attack which was again combated in the same way with the same good result. Strangely enough, there were no further symptoms, whether because some remnant of parathyroid gland had by that time resumed its function or for some other reason, it is difficult to decide.

There seems no doubt that with a suitable emulsion or extract of the ox parathyroid cases of tetany could be successfully relieved for a time but the method of administration is difficult and not without the possibility of unpleasant infection at the site of injection. It would, however, be of great importance to apply this therapeutic test to those forms of tetany which are not definitely known to depend upon the destruction of the parathyroids. It is to be hoped that it may be tested by some one who has the opportunity of studying cases of gastric tetany and the other conditions mentioned above. Especially interesting would be its effect upon osteomalacia and rickets.

The object of our work together this year has been to determine, if possible, the chemical nature of the processes which result from parathyroidectomy and the chemical nature of the functional activity of the parathyroid. The latter part of this problem is far more difficult of solution than the former and we have little to report upon that, but as regards the chemical nature of tetany, we can make a report which, however incomplete, we still prefer to publish now with the hope of communicating the results of further studies later.

Although it was known that some substance contained in the

watery extract of the parathyroid gland, or even in the precipitated nucleo-proteid of that gland is capable of restoring to normal whatever disturbed metabolism we might find in tetany, it was thought best to begin by the investigation of these disturbances before attempting to study the character of this extract. It was proposed, therefore, to place animals under constant conditions for several days before the parathyroidectomy and to observe the chemical characters of the excreta during those days and the whole period following the operation. Further it was thought desirable to study the chemical constitution of the blood, brain, etc., in those dogs which developed tetany as compared with that of normal control animals. The study of the central nervous system was regarded as the more important inasmuch as experiments described in a previous paper[10] seemed to show fairly clearly that the tetanic condition is not an affection of the muscles, but rather of some portion of the brain. In all this it was thought that the study of the mineral contents of the tissues and excreta might be especially important and in view of recent literature on allied conditions it seemed especially important that we should turn our attention to the calcium content. This recent literature which has been well reviewed by Bogen[11] is not extensive and has led so far, to conclusive proof of only a few points. Briefly the papers which bear directly upon the subject are as follows:

The earlier work of Forster[12] and Voit[13] upon the resorption of calcium and its significance in the animal body with the effects of deprivation of calcium in the food gave results of fundamental importance upon the general relations of calcium but gave very little suggestion as to any connection with muscular or nervous hyperexcitability. Pædiatricians had described the nervous and muscular phenomena in infantile tetany and in 1900 Gregor[14] suggested that something in the nature of the nutriment might be the underlying cause of these changes. This was followed later by many more interesting observations on the relations between the nutriment and the development of tetany which will be detailed in order.

In 1901, Sabbatani[15] observed that calcium salts had an action antagonistic

[10] MacCallum, *Medical News,* 1903, lxxxiii, 820.

[11] Bogen, *Monatschr. f. Kinderheilk.,* 1907, vi, 228.

[12] Forster, *Zeitschr. f. Biol.,* 1876, xii, 464; *Archiv f. Hygiene,* 1884, ii, 385.

[13] Voit, *Zeitschr. f. Biol.,* 1880, xvi, 55.

[14] Gregor, *Archiv f. Kinderheilk.,* 1900, xxix, 95.

[15] Sabbatani, *Rivista sper. di Freniatria,* 1901, xxvii, 946.

to that of sodium citrate which he regarded as a "chemical" and not a "pharmacological" action. These effects which concern the irritability of the nervous system are especially marked when the salts are applied directly to the surface of the brain and are as follows: when calcium chloride is applied thus to the cortical surface the irritability as estimated by mechanical or electrical stimuli is almost immediately reduced so that a much greater electrical stimulus is required to produce muscular contraction than in the case of the normal brain. The application of such substances as sodium citrate, sodium oxalate, sodium soap, etc., has the opposite effect, and greatly increases the excitability of the nervous system so that the stimuli necessary to produce muscular movements are now greatly decreased—indeed under these circumstances spontaneous twitchings and even epileptiform convulsions may occur. Subsequent application of the calcium salt will neutralize these effects and reduce once more the excitability of the nerve cells. One can apply sodium citrate on one side of the cortex and calcium chloride on the other with corresponding results on the two sides. All this is due, according to Sabbatani, to the immobilization of the calcium which has a permanent moderating function in the cortex and he suggests that it might be useful in epilepsy but his experiments in this regard were not always successful.

Jacques Loeb[16] in a paper on the production of muscular twitchings by electrolytes, finds that the injection into the animal body of any salt liable to precipitate calcium produces twitching of the muscles, and suggests that "abnormal conditions might arise in which an increase of such acids in the circulation could diminish the amount of calcium in the body. The necessary outcome would be muscular twitchings. In that case the administration of calcium salts might cure the disease." In the suppression of neurogenic twitchings, he thinks it possible that more calcium may be required than in the case of myogenic twitchings. The experiments recorded show that the immersion of a muscle nerve preparation in a solution of a salt which precipitates or renders inactive the calcium, gives rise to twitchings which are suppressed by the addition of fresh calcium to the solution or the transfer of the muscle to a calcium solution. Similarly J. B. MacCallum, in numerous papers,[17] has pointed out that peristaltic activity in the intestine and secretory activity in other organs may be stirred up by certain salts which have an action antagonistic to that of calcium and suppressed by the addition of a soluble calcium salt.

Roncoroni,[18] in 1903, confirmed Sabbatani's work and found that it is difficult to stimulate the cortex after having given intravenous injections of calcium chloride, but that injections of sodium citrate heighten its susceptibility. Histological study of the portions of the cortex treated in Sabbatani's method with various salts show only such modifications as might be due to chemical alterations.

Thiemich[19] described, in 1903, in detail the changes in electrical excitability in cases of infantile tetany which were already well enough known, but pointed

[16] Loeb, University of Chicago Decennial Publications, 1902, x, 6; *Amer. Jour. of Physiol.*, 1900, iii, 327; Garrey, *idem.*, 1905, xiii, 186.

[17] MacCallum, J. B., *University of California Publications, Physiol. Department*, 1903–1906; *Pflüger's Archiv*, 1904, civ, 421.

[18] Roncoroni, *Rivista sper. di Freniatria*, 1903, xxix, 157.

[19] Thiemich, *Rev. d'hygiene et med. infantiles*, 1903, ii, 309.

out also that it is especially in artificially fed children that such tetany occurs, and that cow's milk is especially likely to bring on this condition. Japha[20] similarly recognized, in cow's milk, an important cause of digestive disturbances and of tetany, for its withdrawal and the substitution of mother's milk or farinaceous material brings about a cure. He expressed the opinion that the influence of nourishment is a secondary result of a functional organic disturbance. Finkelstein[21] expresses very similar views in his text-book. Stoeltzner[22] met with similar cases of tetany and tried to analyze the possibilities as to the component of the cow's milk which could thus accentuate or even cause the tetany in children. The result of his experiments is that he regards the tetany as the symptom of excessive retention of calcium in the tissue—a sort of calcium poisoning probably due to intestinal disturbances which prevent the proper excretion of calcium by way of the intestine. As is pointed out by Bogen and others, however, the proof of this theory which Stoeltzner brings forward is not very convincing.

Quest,[23] interested in the work of Sabbatani and Regoli, thought it necessary to determine the calcium content of the brain in convulsive diseases and its relation to calcium metabolism in general. He found that the calcium content of the brain is high in the fœtus and new-born babies but sinks gradually with age and irritability is normally less in the cortex of the new-born than later. In tetany the calcium content of the brain is remarkably lowered. He also tried to produce electrical hyperexcitability in animals by giving them a calcium-free food with the results that the excitability of the calcium-starved dogs is remarkably increased. Weigert,[24] who had an opportunity to study two puppies of the same litter one of which suffered an attack of tetany when fed on cow's milk found that the calcium ratio in the brain was very much diminished as compared with that of the normal dog.

Cybulski[25] studied the metabolism in a child with tetany and does not agree with Stoeltzner's idea, for he found that the calcium retention was far less during tetany than during recovery.

Silvestri[26] estimated that the calcium contents of a fœtus increases rapidly toward the end of term. This must be a great drain upon the mother especially during that time. So too, the amount of calcium secreted in the milk is very high for it amounts to about 0.4 per cent. of the milk and from 1700–1800 grms. are secreted daily which would give an amount of calcium of about 6–7 grms. per day. He thinks tetany is due to a hypocalcification of the nervous centers.

Netter[27] observed good results follow the administration of calcium salts per os in three cases of tetany and thinks that Stoeltzner's views may be recon-

[20] Japha, *Berl. klin. Woch.*, 1903, xl, 1126.

[21] Finkelstein, Lehrb. der Säuglingskrankheiten, 1st half, 1905, Berlin.

[22] Stoeltzner, *Jahrb. f. Kinderheilk.*, 1906, lxiii, 661.

[23] Quest, *Jahrb. f. Kinderheilk.*, 1905, lxi, 114; *Wien. klin. Woch.*, 1906, xix, 830.

[24] Weigert, *Monatschr. f. Kinderheilk.*, 1906, v, 457.

[25] Cybulski, *idem*, 1906, v, 409.

[26] Silvestri, *Gazz. d. ospedali*, 1906, xxvii, 1005.

[27] Netter, *Comp. rend. d. l. Soc. d. Biol.*, 1907, lxii, 376.

ciled with the experimental results of others on the ground that in cow's milk one is dealing with relatively enormous doses of calcium.

Iddo and Sarles are cited by Netter as having found an increased amount of calcium in the urine of a child suffering from tetany which they regard as evidence of an increased excretion of calcium during the tetany.

v. Pirquet[28] opposes Stoeltzner's ideas also on the ground that the hyperexcitability in tetany is by no means dependent upon excessive retention of calcium since it sometimes begins when the amount of calcium taken in with the food is minimal and disappears during the administration of larger amounts of calcium. Bogen also disagrees with Stoeltzner in his idea that an excess of calcium is responsible for the tetany. Other things than cow's milk may induce tetany, the administration of calcium, even in Stoeltzner's hands, did not always result in tetany and after long and harmless administration of calcium acetate, cow's milk is sometimes found to bring on tetany. In seven patients of his own, Bogen[29] found that the administration of calcium will not bring on the tetanoid condition but tends to reduce the excitability of the nervous system and that there is no basis for the belief that tetany arises from a calcium stasis in the organism.

So far for the literature bearing directly upon these relations. Other papers treat indirectly of the same thing, thus several authors among whom Verstraeten and Vanderlinden[30] may be mentioned, make the statement that it is more difficult to produce tetany in animals fed exclusively upon milk than in those given a meat diet. The extent to which this is true will be discussed further on in this paper.

Erdheim[31] has recently attempted to show that there is a direct interdependence between osteomalacia and lesions of the parathyroid bodies and others (Kassowitz) have thought that rickets might be the underlying basis of the tetany of children. Both of these diseases in the course of which tetany is well known to occur are conditions in which there is obviously some profound disturbance of calcium metabolism and the suggestion of their relation to the parathyroid glands at least suggests the study of the relation between the parathyroid glands and calcium metabolism. Erdheim, in his experiments on the production of tetany in rats found most extraordinary changes in the teeth of the rats which

[28] v. Pirquet, *Wien. med. Woch.*, 1907, lvii, 14.

[29] Bogen, *loc. cit.*

[30] Verstraeten and Vanderlinden, *Mem. couronnés de l'Acad. royale de med. de Belgique*, 1894, xiii, 1.

[31] Erdheim, *Sitz. d. k. Acad. d. Wiss., Wien, Math. Naturwiss. kl.*, 1907, cxvi, iii, 311.

became fragile and opaque and frequently broke off short—again some disturbances of however obscure a character in the calcium metabolism.

Our own experiments fall into two groups, in one of which we endeavored to ascertain in cases of outspoken tetany produced by parathyroidectomy the effect of the administration of various substances chiefly mineral salts which might occur normally or under pathological conditions in the animal body and among these we were especially interested in the soluble calcium salts. The second group of experiments was arranged for the study of the changes in metabolism during tetany and in the chemical composition of the tissues in animals dying in that condition.

The effect of the administration of various body fluids and tissues has been mentioned in previous papers but no clear results can be deduced from these. It was shown that the transfusion of blood from a normal dog will suppress the symptoms of tetany when a sufficiently large amount is introduced. Emulsion of brain substance was injected in the same way but the effect upon the tetany was so indefinite that it was questioned in that paper whether the action of the emulsion was any more marked than would have been that of so much salt solution or emulsion of any other organ from a normal dog. Emulsions of the parathyroid gland seem to have a definitely specific effect but that appears only after the lapse of some hours whereas the effect of bleeding and infusion of relatively large amounts of salt solution or blood (200 to 400 c.c.) from a normal dog has an almost immediate effect in supressing the tetany. We could find no other explanation of this phenomenon than that some poisonous material, not destroyed or present in larger amounts than physiologically, on account of the absence of the parathyroid glands, was circulating in the blood. We could not demonstrate the presence of this poisonous substance by injecting the blood from dogs in tetany into normal animals but it may readily be imagined that it is some substance easily oxidized or otherwise changed into harmless materials in the normal body but circulating in the parathyroidectomized dog on account of the lack of some ferment-like material produced normally by the

parathyroid. The idea of a circulatory organic poison we have kept in mind throughout our subsequent experiments.[32]

Our attention was turned then to the inorganic substances which might be concerned. Salts of sodium, potassium, calcium and magnesium are of course prominent. We began with the effects of calcium subsequently experimenting with the others as follows:

2107. Thyroparathyroidectomy, four days later violent twitching of muscles—pulse, 160, respiration labored. Given 10 c.c. of a 5 per cent. solution of calcium acetate into jugular vein. Respiration became rapid, 200 to minute, twitching rare but sharp—twenty-five minutes after the injection, pulse was 80, very irregular and slow. Dog thought to be dying—occasional slight twitches. Next day dog was found walking about and fairly well but was found dead the day after. In this experiment the animal was apparently restored to life from a moribund state, but the amount of Ca salt had not been large enough to remove tetany instantly.

2207. Four parathyroids extirpated December 20. On December 22 at 3 P. M. most violent tetany, with tachypnœa, respiration 200, pulse 132, temperature 40.75. December 22 at 3.05 P. M., given 10 c.c. of 5 per cent. solution calcium acetate into jugular vein. 3.10, respiration 240–250, twitchings intense with snapping of teeth. 3.25, tachypnœa continues irregularly at a rate varying from 160–170. 3.30, breathing much quieter but still unnaturally rapid. No panting at present, twitching markedly improved, pulse 100. Dog now lies quietly and is fairly well relaxed. Twitchings very slight, felt only in shoulder. Lifts up his head and wanders about, is breathing quietly and seems comfortable—respiration 100, twitching has practically disappeared. 3.35, respiration 80, pulse 95—dog now quiet and takes intelligent interest in surroundings. 3.40, respiration 35, pulse 90, slight twitching. 3.45, respiration 21, dog resting quietly—apparently rather exhausted. 3.50, runs about actively, swaying slightly with an occasional jerk of one leg, but on the whole one could not tell that he had had tetany. Responds eagerly to petting and eats greedily. 6.30, seems quite well—no trace of twitching—is very quiet and tractable, no distinct tetany. December 23.

108. January 9, two parathyroids and one lobe of thyroid extirpated—no results. January 18, second lobe of thyroid removed. January 19, violent tetany—at 11.30 given 10 c.c. of 5 per cent. calcium lactate subcutaneously. 11.35 respiration 240, marked twitching, pulse 180, breathing very rapid and labored. 12.10, still tachypnœa and twitching. 1.10, respiration slowed, 40 to minute, muscular twitching still marked. 1.30, respiration quiet, 1.32, twitching has almost disappeared, slight muscular tremor, walks about but looks dejected. 3 P. M., respiration 24, pulse 124, dog is quite normal in appearance, no tremor nor twitching. Respiration perfectly quiet and animal has perfect control of himself and eats hungrily on being taken to cage.

It is seen from this experiment that the effect of subcutaneous injection of calcium is the same as that of intravenous injection

[32] MacCallum and Davidson, *Medical News, loc. cit.*

but appears more slowly. The dog developed tetany again next day and his further history will be given in connection with experiments with potassium salts.

208. Thyroparathyroidectomy, January 16. On January 18, at 9 A. M., found in violent tetany; tachypnœa, twitching and loss of control of legs, unable to stand. 11.30, 80 c.c. of 5 per cent. solution of calcium acetate given by stomach tube; after a short time was able to walk and tetanic spasms disappeared, but animal was found dead next day.

2208. This was one of Dr. Halsted's dogs from which he had removed the thyroids and parathyroids after having transplanted one or two parathyroid glands into the abdominal wall. After the total removal of the glands in the neck the dog developed tetany, since at that time the transplanted parathyroids were evidently not functioning actively enough to prevent it. February 14, dog is having marked tetany, at 5 P. M. respiration very rapid, pulse 120; given 100 c.c. of 4.3 per cent. calcium lactate by stomach tube. 6.30, respiration still rapid, but tetany has stopped. February 15, 10.15 A. M., dog is fairly well, but has fibrillary twitchings in tongue, respiration is rather deep and rapid, 110, pulse 100. Passage of the stomach tube elicits an attack of tetany with respiration at 304 and very rapid pulse. 10.45, 100 c.c. of 4.3 per cent. calcium lactate given by tube. . 11.00, respiration 60, deep and regular—at 2 P. M. no tetany could be observed. February 17, 9 A. M., dog is having tetany—given 50 c.c. of 4.3 per cent. calcium lactate at 10.30; in afternoon was all right. February 18, no tetany, but dog was given during the day two doses of calcium lactate as a prophylactic measure. February 19, no tetany, given 40 c.c. of 5 per cent. calcium acetate. February 20, no tetany, given 50 c.c. 5 per cent. calcium acetate. February 21, no tetany. February 25, slight twitchings of muscles of head; given 100 c.c. of 4.3 per cent. solution of calcium lactate by stomach tube. March 3, no tetany.

This dog gradually developed very distinct myxoedema but up to the present, July 3, there has been no further sign of tetany. Evidently the condition which led to the tetany was tided over by the administration of calcium until the transplanted parathyroid became able to function actively enough to prevent tetany.

2508. February 27, parathyroidectomy. February 29, 9 A. M., marked tetany, pulse 128, respiration 36. 11.40, tachypnœa. 11.48, 10 c.c. 5 per cent. sodium acetate intravenously. Respiration 150, pulse 160. 11.50, rigidity, twitching increasing. 12, tachypnœa, violent twitching, no improvement whatever. 12.10, pulse 156, respiration 240, temperature 40. 12.15, 10 c.c. of 5 per cent. calcium lactate intravenously. 12.22, respiration 252, pulse 136, tetany intense. 12.30, 10 c.c. of 5 per cent. calcium lactate intravenously. Tachypnœa intermittent, throughout some periods of 5 seconds respiration is at 300, then it falls to almost nothing, starting quickly again. The muscular twitching is almost gone. 12.35,

fibrillary twitching of the tongue almost gone, muscles flaccid, practically no twitching, except in the superficial muscles. Respiration 132, not labored. Dog begins to take interest in the surroundings, respiration 132. 12.45, respiration 100, 5 c.c. calcium lactate intravenously. 12.50, respiration 60, dog seems practically normal and is allowed to run about the floor. During the next three days twitching was very slight and one dose of calcium lactate was given on March 1, large quantities of milk, 500 c.c. to 1 l., March 2, 3, 4. March 5, dog was found in violent tetany, giving 400 c.c. of milk, 10.30 A. M. At 11.10 an epileptiform convulsion occurred in which he fell to the floor, rigid and breathing stertorously. This lasted a minute or two, when he got up, shaking himself, walked away. 2 P. M., still twitching violently, respiration labored and grunting. 3 P. M., given 400 c.c. of milk by stomach tube. Passage of the tube throws the dog into an epileptiform state, in which he lies rigid on the ground with grunting respiration. Apparently the quantity of calcium administered in the milk is far too small or is not absorbed readily enough to cure or ward off tetany. Up to 5.30 P. M. still having tetany. March 6, still is stiff and twitching, but not in violent tetany. 12.30, given 400 c.c. milk. Violent spasm during introduction of tube. 6 P. M., 300 c.c. more of milk, tetany is slight. From March 7 to May 4 the dog was left out in the yard, apparently well enough, with no tetany. He was on a meat and bone diet. May 4, found in violent tetany, almost moribund and given calcium lactate by mouth and left to himself. May 5, dog is apparently normal. Up to May 15 he was quite well, when he was found in a violent attack of tetany, given 100 c.c. of 4 per cent. calcium lactate by mouth; at 6 P. M. is in violent tetany with rapid, labored respiration. Legs rigid and twitching. Respiration 128, pulse 168. At 6.20 is still in tetany. At 8 P. M. is well, can walk about, but occasional twitches in the muscles. Respiration 40, pulse 152. Dog seems pretty comfortable and it was confidently expected that he would be all right in the morning. May 16, perfectly well and remained so up to June 7, when another attack of tetany was suppressed in the same way by giving calcium lactate by mouth. Since that time he has been well up to July 15, when he was killed by ether.

3708. Another dog operated upon by Dr. Halsted for transplantation of the parathyroid gland with subsequent extirpation of all of the remaining parathyroids, was cured of several attacks of tetany by doses of calcium lactate given by mouth.

6008. Operated upon March 17, transplantation of one parathyroid into abdominal wall. Second operation May 5, transplantation of another parathyroid into abdominal wall and removal of all parathyroid and thyroid glands in the neck. May 6, 4 P. M., violent tetany, given 50 c.c. of 5 per cent. calcium chloride by mouth. May 7, slight tetany, given 50 c.c. 4 per cent. calcium lactate by month. One hour later tetany had disappeared. May 8, no tetany. May 9, slight tetany; in the morning given 50 c.c. of calcium lactate 5 per cent. by stomach tube; complete recovery. May 10, twitching on the top of the head, given 50 c.c. 5 per cent. calcium lactate. May 11, having distinct tetany with snapping jaws, given 100 c.c. of calcium lactate. May 12, no tetany. May 13, slight tetany, given 100 c.c. 4 per cent. calcium lactate per os. May 16, slight

tetany, given 100 c.c. 4 per cent. calcium lactate per os. Since this time the dog has had no further tetany and has received no further calcium.

These experiments are sufficient to demonstrate the immediate and specific curative effect of the administration of soluble calcium salt upon the tetany of parathyroidectomy, the specificity being shown by experiments which will be recorded in which the effect of calcium is striking as compared with the inefficiency of other salts in suppressing tetany. It is seen from these experiments that the effect of calcium adminstration is quite similar, no matter whether it be given intravenously or subcutaneously or by stomach tube. The rapidity with which it acts differs, however, under these different conditions. It is found that the calcium chloride is, perhaps, the most irritating of these salts and cannot well be administered subcutaneously. Furthermore, the too rapid injections of calcium lactate into the venous channels may give rise to hemorrhages in the pulmonary tissues. We have, however, observed no other ill effects in any of the animals even when very large doses of calcium were administered rapidly.

At this point it may be stated that an opportunity has occurred for the observation of the effect of calcium upon cases of tetany in human beings produced by the operative extirpation of the thyroid together with the parathyroids on account of tumor growths, etc. Dr. J. H. Musser, of Philadelphia, has been kind enough to allow us to see a case under his charge, in which violent tetany had developed as the result of the operative extirpation of an extensively invading malignant growth of the thyroid gland. In this case calcium lactate was administered in frequent and quite large doses with the result that tetany disappeared in the course of one day. When the calcium medication was withheld for two or three days, the symptoms of tetany reappeared, only to disappear on the readministration of the calcium. In a portion of thyroid removed at operation in this case we have been able to find only one parathyroid gland, but the tissue was so much cut up, and such considerable portions had been removed, that the examination can make no pretense to completeness. A similar case has been described to us by Dr. S. J. Meltzer as occurring in the practice of

a New York physician, in which the administration of calcium salts suppressed the symptoms of tetany in precisely the same way.[33]

Dr. Meltzer in recent papers has pointed out the antagonistic action which exists between calcium and magnesium salts, and has further emphasized the anæsthetic action of magnesium salts and their importance in modifying the symptoms of tetanus when injected about the central nervous system. We have, therefore, been interested in observing the effects of magnesium salts upon the symptoms of tetany, and have made a few experiments as follows:

2207. Referred to above. In this dog in which tetany first developed on December 22 a cure was brought about by the injection of calcium, but on December 24 he was again found in moderate tetany, twitching very distinct, legs very stiff and as feet continually double under him, he cannot easily stand, respiration 24, labored, pulse 144, temperature 38. At 10.35 given 10 c.c. of magnesium chloride intravenously, breathing at once became very quiet. Tetany practically stopped. The twitching is still visible, however, in the shoulders and face. 10.40, respiration 35, pulse 100. General slight tremor, but limbs are relaxed. 10.45, respiration 44, more labored, twitching much more marked, legs stretched out. 10.55, twitching just evident. Dog is very apathetic, stands with head hanging, makes slight attempts at vomiting. He is perhaps quieter and more comfortable than before the injection was given, responds slightly to a call. 11 A. M., twitching scarcely visible. Dog still stands most dejectedly with hanging head. 11.45, cowers in the corner, stiff and scared, slight tremor, breathing audible across the room. No marked tetany. 2 P. M., slight twitching, dog has anxious look and attempts to remain in a dark corner, no definite tetany. 5.30, dog was stuporous but there was no distinct twitching and no definite tetany for three days following this, when tetany began to appear and was suppressed by injection of calcium salt.

1508. February 8, thyroparathyroidectomy, one lobe of the thyroid being left. February 10, 9.30 A. M., dog in violent tetany, pulse 152, respiration very irregular with periods of apnœa. 10.45, given intravenously 10 c.c. 5 per cent. magnesium chloride. Dog stopped breathing, but was kept alive by artificial respiration, the pulse continuing fairly strong. After a time the dog began to breathe himself, but very slowly. At 11 o'clock respiration 28, pulse 132. Dog seems to be completely anæsthetized, does not move with the prick of a pin and there is hardly any corneal reflex. Tetany completely stopped. At 11.10 still perfectly unconscious and breathing quietly. 11.30, walking about but is

[33] Dr. Harvey Stone, of Charlottesville has described to us in a recent letter the case of a little girl suffering with what was apparently gastric tetany, the symptoms of which have been completely relieved by the administration of calcium. While this tends to give some support to the idea that gastric tetany is dependent upon a relative insufficiency of the parathyroid gland, the effect of the administration of parathyroid extract in such a case would be still more interesting. Dr. Stone will shortly report the case and kindly permits us to mention it here.

very ataxic. 11.50, very apathetic, lies shivering on the floor, no tetany. 5.30, in the afternoon, the dog still lay curled up in the cage, could hardly be roused. February 11, 9.30, still lies curled up without distinct tetany, but next day, February 12, tetany appeared in a most intense form.

Unfortunately up to the present, we have not made any further experiments with magnesium, but these seem to indicate that the injection of magnesium will suppress the symptoms of tetany, though its effects are somewhat confused by the ataxic and anæsthetic action of the magnesium itself. Whereas Dog 1508 was extremely stuporous the next day after the injection, another dog suffering quite severely from tetany at the same time received instead of the magnesium an injection of calcium salt, and the next day was to all appearances perfectly normal.

A few experiments have also been made on the effects of sodium and potassium salts in relation to tetany as follows:

108. Tetany developed January 19 and was suppressed by a dose of calcium given subcutaneously. Next day, January 20, tetany was again quite marked, the dog rendered helpless. 11 A. M., respiration grunting 40 to the minute. At 11 A. M. 10 c.c. of 5 per cent. potassium acetate given subcutaneously. At 11.20 tetany, if anything, slightly more marked than during the morning, animal stretched out helpless, breathing rapid and labored. Later in the afternoon when he seemed practically moribund, he was given 10 c.c. of calcium lactate, 5 per cent. solution, subcutaneously and still later 10 c.c. intravenously; was apathetic for the rest of the afternoon, but fairly well next morning. In this case the potassium acetate seemed to have no controlling influence over the tetany.

808. January 23, four parathyroids removed. January 26, distinct tetany with twitching, very violent. Legs rigid, pulse 148, respiration very irregular. 9.50, given 10 c.c. 5 per cent. potassium acetate subcutaneously. 10.10, tetany quite violent and at 3.30 distinct violent tetany, respiration labored. 10.45 P. M., marked tetany, pulse 160, respiration 28. As his condition seemed to be very serious 10 c.c. of 5 per cent. calcium lactate was given subcutaneously. Tetany still very distinct at 10.55. At 11.45 pulse 76, extremely forcible. At 11.50 tetany had disappeared and the dog walked about and behaved quite normally. January 27, at 6 P. M. dog which had been somewhat apathetic all day again developed tetany. At 8.30 pulse 176, respiration 36, given 10 c.c. calcium lactate intravenously, pulse immediately afterwards 56, respiration 28, tetany stopped almost immediately. January 27, no tetany. January 28, 10.30, slight but distinct tetany. 10.45, given intravenously 10 c.c. 5 per cent. solution sodium acetate. Almost immediately the pulse became uncountable, respiration from 100 to 120. Tetany at once become violent. 10.55, tetany extreme. Dog is unable to rise but lies on its side with legs stretched out, twitching violently, fibrillar tremors of the tongue, rigidity of the legs renders the animal help-

less. This continued until 12.15 when 8 c.c. of calcium lactate was given intravenously; at that time the pulse was 176, respiration 100 to 120; within a few minutes the tetany completely disappeared. Pulse rate reduced to 76, respiration 36. The dog can walk about and takes an interest in things, drinks and eats. At 2 P. M. tetany reappeared to a slight degree. At. 2.20, 10 c.c. calcium acetate were injected subcutaneously. At 2.45 another 10 c.c. At 4.50 the dog seemed well enough. The tetany reappeared on each of the next two days, but was relieved almost at once each day by the intravenous doses of calcium lactate.

1908. February 7, complete thyroparathyroidectomy. February 10, dog found in violent tetany with tachypnœa, respiration 168, pulse uncountable. At 11.25 given 5 c.c. of 5 per cent. potassium acetate intravenously. 11.32, respiration 288 to 300, pulse not counted, twitchings are evidently more violent than before. Pulse at 11.40, 144, tachypnœa and twitching very violently, fibrillary contractions of the tongue, jaws clenched. 11.45, respiration 270, very labored. 11.50, respiration 300, very irregular and in periods of violent tachypnœa. After this, was given a number of doses of calcium lactate intravenously 5 c.c. at a time until one o'clock when it became quiet and the dog could walk about. At 5.30 P. M. the dog seemed perfectly well and remained so until February 13, when he was found again in moderate tetany. At 10.38, 10 c.c. of 5 per cent. sodium acetate was given intravenously which was found to have no effect whatever upon the moderate tetany. At 4 P. M. 40 c.c. calcium lactate was given by a stomach tube and tetany gradually disappeared, being quite absent next day. The dog died two days later of penumonia.

The experiments with the injection of potassium salts are unfortunately very meagre and unsatisfactory, but we hope to repeat them later with more definite results; at least the injection of potassium salts seems to exercise no definite beneficial influence upon the course of the tetany, and in case 1908 it seemed necessary to administer more calcium than usual in order to overcome the tetany. Whether this was due to the previous injection of potassium salt or not cannot be definitely stated.

The experiments were especially unsatisfactory on account of the fact that in the hope of saving the dog's life, calcium was injected in almost every instance before sufficient time had elapsed to determine the exact effect of the potassium injection. The same thing is true in a case of sodium injection in Experiment 2508 recorded above. The studies of metabolism, to be detailed later in this paper, showed that in the course of tetany the ammonium content of the blood and of the urine is somewhat increased and the idea has been prominent that possibly something resembling an acid intoxication might be responsible for the symptoms. We have,

therefore, planned the administration of large doses of sodium bicarbonate to see if this hypothesis be true and to ward off the development of tetany. Unfortunately these experiments are as yet incomplete, but such as they are may be recorded here.

4808. Complete thyroparathyroidectomy April 25. April 26, given 25 gms. sodium bicarbonate, dissolved in water, by stomach tube. April 27, no symptoms, given 50 gms. of sodium bicarbonate by stomach tube. April 29, no twitching, no tremor of tongue, given 25 gms. sodium bicarbonate; dog was found dead next day without having developed any symptoms of tetany.

4908. April 25, complete thyroparathyroidectomy. April 26, given 50 gms. sodium bicarbonate by stomach tube. April 27, no distinct symptoms of tetany, given 50 gms. sodium bicarbonate by stomach tube. The passage of the tube elicited a sort of epileptiform attack, in which the dog quickly recovered. April 28, no symptoms. April 29, slight twitching of the muscles of back and of the tongue, given 25 gms. sodium bicarbonate by stomach tube. 6 P. M., distinct tetanic twitching about head. May 6, dog has shown no tetany during the past six days. Died subsequently without having shown any such symptoms.

5708. May 14, four parathyroids removed. May 16, 6 P. M., distinct signs of tetany. 11.15 P. M., tetany quite violent, respiration 280, legs very stiff, pulse 156. 11.30, great rigidity, extreme tachypnœa, pulse 230. 12.00 midnight, pulse 168, respiration 240, occasionally twitching in hind legs, 25 c.c. of 5 per cent. sodium bicarbonate injected into jugular vein, tetany during this more violent than before, respiration less regular, but less continuously rapid, 200 or less. 12.15, respiration 240, twitching still quite violent. 12.30, respiration 112, alternating with brief intervals of 218. 12.35, tetany is reduced but tachypnœa is still very marked, fibrillar twitchings of the tongue, intense retraction of the eye-lids, respiration 248, with rarer intervals of slow breathing. 12.45, is somewhat better, no noticeable twitching, tachypnœa recurs at intervals but stops when his attention is attracted, fibrillar tremors of the tongue persists. 1 A. M., continuous tachypnœa, 240. Dog is very awkward and stiff, conjunctivæ exposed, marked tremors and twitching in the legs, which are moderately stiff. 1.30, still a continuous marked tachypnœa, can walk only with difficulty owing to the lack of control of the legs. May 17, 9.30 A. M., dog seemed perfectly well, no twitching or fibrillary tremors of the tongue and no tachypnœa. May 18, no tetany. May 19, slight transient tetany. After this the dog was practically well until May 29, when he showed slight twitching.

6108. May 18, two parathyroids and one thyroid lobe were removed. May 20, tetany. 11 A. M., marked tetany with tachypnœa. 11.50, 25 c.c. 5 per cent. sodium bicarbonate injected into jugular vein. 11.55, twitching more violent, respiration 220. 12.00, respiration 232, tetanic spasms much more violent, injection of the sodium bicarbonate complete at 12 o'clock. At 12.15 respiration 240, twitchings very violent. 12.30, still in violent tetany with occasional tachypnœa and rigidity extreme. This continued until 8 P. M., during which time the respiration was very labored and twitchings persisted, though they became less violent toward evening. May 21, no tetany, dog is very apathetic and stuporous. May 23, dog found in violent tetany, respiration labored, 52

to a minute, extreme fibrillar twitchings of the tongue. 10.15, 25 c.c. of 5 per cent. sodium bicarbonate injected intravenously. 11.10, the twitching persisted, breathing not so labored. May 24, slight tremor but no distinct tetany. There was no tetany again, dog died June 1, after having lain in the cage in a stuporous condition during that time.

6308. May 19, thyroparathyroidectomy. May 20, marked tetany. At 12.06 respiration 240, violent muscular twitching and rigidity, fibrillar tremors of tongue. From 12.14 to 12.21, 25 c.c. 5 per cent. sodium bicarbonate were injected intravenously. At 12.22 convulsive twitchings were most violent and rigidity was extreme. 12.40, condition is similar but rather worse. 1.20, most violent tetany. 2 P. M., extremely violent attacks of tetany lasting about 5 minutes and succeeded by exhaustion. From 2.00 until 3.15 the twitchings became less marked, the body relaxed. The dog became unconscious; death took place at 3.15.

It is to be seen from these experiments that there is a very striking contrast between the effects of even very large doses of sodium bicarbonate and those of the salts of calcium in those animals in which tetany has already developed. For, while some of the animals recovered and showed no tetany next day, one at least died in the tetany without experiencing the slightest relief, and all of the others continued to show violent symptoms of tetany for hours without any such relief as was invariably observed to follow the administration of calcium.

If we imagine that there is an acid intoxication with the setting free in the tissues of excessive amounts of organic acids[34] which must be neutralized by some alkaline substances withdrawn from the tissue, among which are calcium and ammonium, it seems possible that as far as this is concerned the administration of other calcium or sodium might furnish the necessary material, but the administration of sodium would not replace in the tissues the calcium which has already been abstracted in dogs in which tetany has already developed. A minimum amount of calcium seems to be absolutely necessary for the normal function of the nervous system, and sodium cannot take its place. The administration of sodium directly after the operation, however, might well prevent any such abstraction of calcium from the tissues. More experiments than the two recorded must be performed in order to determine this point. The administration of calcium, however, would in addition to the restoring of the balance, directly restore the

[34] Lactic acid was found in blood in one case.

calcium to the impoverished tissues. Since we know that the injection of such salts as sodium chloride is without definite effects upon the tetany, it is probable that whatever beneficial influence is obtained from the injection of sodium bicarbonate is due to its basic properties after being split into sodium carbonate and carbon-dioxide.

In a number of cases an attempt was made to study the changes in metabolism which result from parathyroidectomy. In order to do this it was thought necessary to reduce as much as possible the calcium content of the food so that any error in the estimation of the amount of calcium excreted might be as small as possible. It was finally decided that the error involved in the possibility that some of the calcium of the food might pass through the intestine without having been absorbed could be eliminated best by causing the animals to fast entirely for several days before the operation, and also after the operation; they were therefore put in metabolism cages, given daily a measured amount of water but no food. Diarrhœa was prevented by the administration of kaoline or kieselguhr (free of calcium) in the water. The dog was catheterized every morning at the same hour, and 24 hour specimens of urine as well as the fæces were collected. It remained then only to analyze the urine and fæces of these dogs with respect to their ammonium content, calcium content, etc. In some of the experiments the dogs were bled at the end of the period of tetany and the calcium content of the blood determined. At autopsy the calcium content of the brain was also determined. The results of these experiments will be more clearly understood from the study of the tables which are given at the end of this paper.

As large a number of dogs as could be was studied in this way, during the time at our disposal, for the process is an extremely laborious one. This is mentioned on account of the fact that successful experiments in our series are so few. So many vicissitudes were encountered and such a large proportion of the dogs thus operated upon proved to have accessory parathyroids in their thyroid glands, that the number of complete and proved experiments is very small. In only three dogs have we succeeded in producing tetany under these conditions, and in obtaining complete records of the

analyses of the urine and fæces. In two others we have analyses of the urine and fæces after a partial extirpation of the parathyroids but without the development of tetany. In another we have the analysis of urine and fæces after the operation for the removal of the parathyroid which was not successful in bringing about tetany but which was followed by the second operation by removal of the thyroid lobes which did result in tetany from a coincident destruction of the remaining parathyroid tissue. The absence of the thyroid gland doubtless modifies the metabolic changes in this case. The analyses of the blood and of the brains were sometimes made from the dogs used for urinary and fæcal analysis, but other animals, both normal controls, and animals suffering from tetany were also used for this purpose. Objection might possibly be raised to the employment of this method of fasting of animals during the experiment, on the ground that in human beings, at least, fasting is very likely to bring about an acid intoxication with changes in the ammonium ratio in the blood and with the excretion of acetone in the urine. Numerous experiments have, however, shown that this does not follow starvation in the dog. In gross the results of the analyses which are detailed in the tables are about as follows: The blood of the dogs which were bled during an attack of tetany shows a content of calcium which is only about one-half of that of the normal dog. The ammonium content is on the other hand enormously increased, and may reach a value ten times as high as that of the normal animal. The analyses of the brain of dogs during tetany, shows the presence of a very greatly diminished amount of calcium; it being not more than about half of that found in the normal brain. The analysis of the urine and fæces should be considered in one series as representing a total excretion of calcium, etc. It is found in general, that the amount of calcium excreted in the urine decreases during the first two or three days of fasting, finally reaching a fairly constant low level. After the operation, as far as our curves indicate, there appears, during tetany a gradual rise in the amount of calcium excreted. The estimation of the total excretion of calcium is rendered difficult by the fact that the passage of fæces is extremely irregular and the calcium actually excreted into the intestines may accumulate

there during several days, so that the construction of an accurate curve is almost impossible. In order to make such a curve more accurate a much longer period of observation is required. It seems possible in future experiments that a fairly accurate idea might be obtained by washing out the intestines every day. On the whole the general impression which was received from these studies is that in the course of tetany, following parathyroidectomy, the excretion of calcium salts in the urine and fæces is somewhat increased, and the tissues of which we have examined the blood and brain, are remarkably impoverished with regard to calcium. In this way the condition described by Sabbatani and Loeb and others appears to be produced and the symptoms of tetany may well be explained on the basis of this impoverishment of calcium in the tissues. The actual amount of calcium in the tissues is so small that the loss of calcium which may appear almost infinitesimal may still be sufficient to overthrow the balance and give rise to the hyper-excitability which one observes in tetany.

It is on this basis, therefore, that the administration of large doses of a soluble calcium salt might be expected to restore the calcium content of the over-excitable tissue and thus to suppress the symptoms produced by this impoverishment. It must be observed that the administration of calcium by no means acts as a cure but merely suppresses the symptoms of a disease, the actual cure of which could be brought about only by the implantation of the parathyroid gland, or temporarily by the injection of an extract of the parathyroid gland. Nevertheless, the relief afforded by the administration of calcium, in cases of tetany, is so complete, and the animal is apparently so nearly normal under the continued administration of calcium, that it may well prove to be a therapeutic agent of some value in tiding over the period of violent tetany until some more permanent cure may be instituted.

The calcium content of the parathyroid glands themselves has been studied and found extremely minute, about equal to that of the voluntary muscle.

The bearing of these results, in so far as they offer an explanation of the various types of tetany mentioned in the beginning, is clear enough in certain classes of cases, but in others, for example

the cases of gastric tetany, it is by no means easy to understand. In osteomalacia and rickets we have evidently a common cause which leads to the decalcification of the bones and other tissues and consequently to tetany. Whether this depends upon a lesion of the parathyroids as Erdheim suggests remains to be determined. In the tetany which accompanies pregnancy and lactation, however, we can easily believe that there is no organic lesion of the parathyroid gland but that the decalcification of the tissues is directly due to the extraordinary drain upon them in the production of the bones and other calcium-rich tissues of the fœtus or in the secretion of milk which has a large calcium content. It would be of interest at least to make a therapeutic test by the administration of large doses of lime water or of any other soluble calcium salt in these cases.[35]

TABLE I.

Calcium Content of 100 c.c. of Arterial Blood.

(Obtained from carotid artery.)

Normal Control Dogs.		Tetany Dogs.	
No. 608	0.0122 gr. Ca.	No. 1108	0.0053 gr. Ca.
No. 3608	0.0140 gr. Ca.	No. 508	0.0065 gr. Ca.
No. 1608	0.0138 gr. Ca.	No. 308	0.0056 gr. Ca.
		No. 2708	0.0046 gr. Ca.
		No. 5708	0.0052 gr. Ca.

Calcium Content of 100 gr. of Dried Brain.

Control Dogs.		Tetany Dogs.	
Cerebrum	0.0841 gr. Ca.	Cerebrum	0.0472 gr. Ca.
	0.0750 gr. Ca.		0.0433 gr. Ca.
	0.0792 gr. Ca.		0.0531 gr. Ca.
			0.0510 gr. Ca.
Cerebellum	0.0560 gr. Ca.	Cerebellum	0.0326 gr. Ca.
Medulla	0.0410 gr. Ca.	Medulla	0.0323 gr. Ca.

[35] Since the publication of our preliminary report we have noticed a review in *Folia Hæmatologica*, 1908, v, 101, of an investigation by G. Parhon and C. S. Ureche, on the influence of calcium and sodium salts upon the course of experimental tetany. This was published in the *Revista Stiintelor Medical*, 1907, July and August, a journal which is unknown to the librarian of the Surgeon General's Library. They produced tetany in dogs by removing thyroids and parathyroids and found that injections of sodium chloride rather increased the symptoms, while injections of calcium chloride tended to quiet them. In both cases, however, the animals died of tetany much more quickly than the controls which had received no injections.

Ammonia Content of Blood.

50 c.c. of blood taken during tetany contain.....0.0051 gr. NH_3.
50 c.c. of blood taken from normal dog contain....0.00206 gr. NH_3.

TABLE II.

Showing Effect of Parathyroidectomy in Dogs No. 5008 and No. 5108 on Nitrogen Metabolism.[86]

Methods used for metabolism experiments were as follows:

Total nitrogen.....Kjeldahl's method.
Ammonia.....Folin's method.
Chlorides.....Titration with ammonium sulphocyanide.
Phosphates.....Titration with uranium acetate.
Creatinin.....Folin's method.

Calcium was determined as follows: Incineration of organic material, precipitation of dissolved ash with ammonium oxalate and titration of oxalic acid contained in resulting calcium oxalate with potassium permanganate. This method gives accurate results, while the amounts of calcium concerned were too small for accurate estimation by gravimetric methods.

Date.	No. 5008.				No. 5108.			
	Volume of Urine, c.c.	Total Nitrogen in gr.	NH_3-Ratio.	Remarks.	Volume of Urine, c.c.	Total Nitrogen in gr.	NH_3-Ratio.	Remarks.
May 2	185	2.51	7.26		265	3.92	6.34	
3	355	4.31	7.28		420	3.37	5.40	
4	300	3.59	7.02		320	2.40	18.41	Operation.
5	305	4.35	10.91	Operation.	325	2.43	41.84	
6	420	4.65	6.82		270	2.94	25.45	
7	315	3.54	9.21		435	3.81	20.94	
8	375	5.00	4.51		430	4.90	23.58	
9	305	4.61	4.77		210	7.61	13.63	Tetany violent.
10	365	4.44	7.14		245	4.81	29.58	Tetany slight.
11	430	2.98	10.33	Tetany slight.	255	4.86	37.31	Tetany slight.
12	475	5.99	12.00		250	5.10	33.00	Tetany slight.

Explanation of Table II.—Notice rise in total nitrogen and ammonia ratio in dog No. 5108. This animal had no parathyroids left after operation and began to show tetany May 9, and was found dead May 13. With onset of tetany volume of urine is decreased very markedly.

In dog No. 5008 operation was not altogether successful, as some parathyroid tissue was left behind. Insufficiency produced changed composition of urine to a less extent than in No. 5108. Dog showed tetany on one day only and recovered afterwards to live for a number of days without any apparent discomfort, when the thyroid was extirpated, and tetany appeared in a violent form, showing that some interior parathyroid which had not been removed at first operation was extirpated together with thyroid.

[86] *General characters of the urine after the appearance of tetany.*—The urine shows a high specific gravity and is correspondingly decreased in amount. There is a cloudy precipitate, no albumen, acetone, diacetic acid, nor sugar could be detected in any of the cases.

TABLE III.

Female Dog No. 1807. Weight 21.1 Kilos. Was Fed for the First Five Days on 800 c.c. of Milk Daily. Then Operated for Parathyroidectomy and Starved. Gets only 800 c.c. of Water now. Composition of Urine:

Date.	Volume, c.c.	Spec. gr.	Total nitrogen.	NH_3—N	NH_3 ratio.	Ca	NaCl	P_2O_5	Creatinin.	Total Acidity.
Dec. 19	710	1036	4.73	0.86	8.00	0.079	21.3	1.17	0.71	2.30
20	785	1024	5.77	0.39	6.88	0.030	17.6	0.95	0.46	1.25
21	555	1024	4.16	0.26	6.35	0.026	6.1	0.48	0.29	.44
22	410	1020	2.53	0.10	4.06	0.011	4.3	0.25	0.23	.20
23	490	1032	6.42	0.65	10.2	0.035	5.9	0.78	0.46	.49
24	350	1033	7.20	0.67	9.30	0.036	6.5	1.36	0.42	.53
25	355	1030	6.80	0.70	10.29	0.041	7.5	1.40	0.55	.59

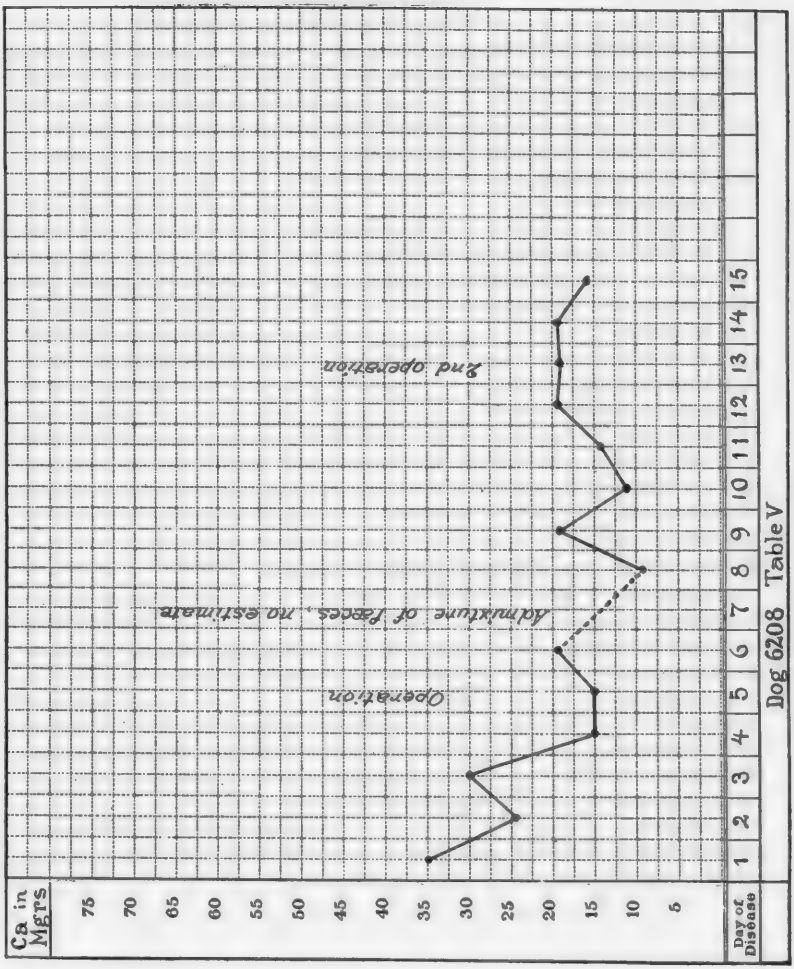
Explanation of Table III.—Dog was operated December 22. Violent tetany was observed December 24 for first time. Had several severe attacks and was found dead December 26. No fæces were obtained. Before operation animal adjusted metabolism to the low diet. From day to day less is excreted with urine. After operation all of the constituents determined are found to be present in larger quantities, in spite of the fact that animal is starving.

TABLE IV.

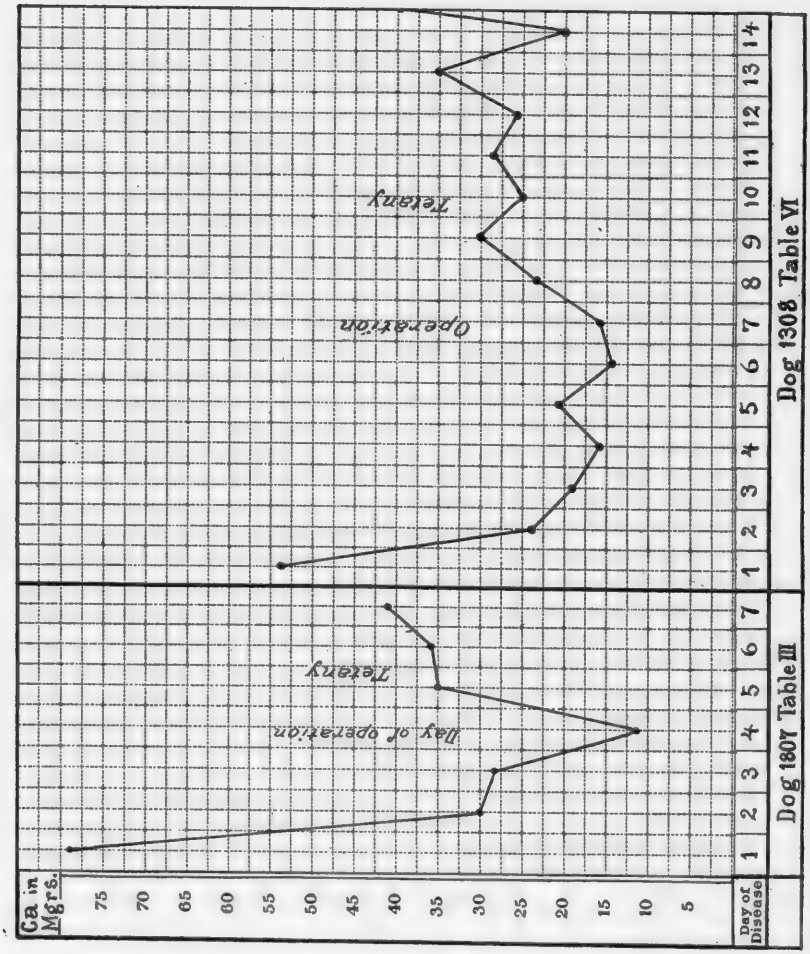
Female Dog No. 7208. Received 400 c.c. Water Daily.

Date.	Urine.		Fæces.
	Volume, c.c.	Ca in gr.	Ca in gr.
June 24	254	0.0081	
25	240	.0086	0.3279
26	265	.0098	2.5450
27	254	.0085	
28	248	.0083	1.8722
29	258	.0092	
30	300	.0092	
July 1	300	.0052	
2	230	.0061	0.7882
3	305	.0057	
4	295	.0108	
5	365	.0092	
6	285	.0087	
7	245	.0084	
8	230	.0082	

Explanation of Table IV.—This animal was kept as a control under absolutely the same condition as the ones on which parathyroidectomy was performed. Variations in volume of urine are very slight. Calcium excretion with the urine remains on about the same level with the exception of three days (June 1, 2 and 3), when calcium output is reduced approximately 30 per cent. The calcium of the fæces is decreased considerably during the course of the experiment.



CURVE I.—The curve illustrates Table V, Dog 6208, in which parathyroidectomy was incomplete and did not result in tetany.



CURVES 2 AND 3.—Curve 2 illustrates Table III, Dog 1807. Curve 3 illustrates Table VI, Dog 1308. Both curves show the variation in the calcium output in the urine before and after the development of tetany.

TABLE V.

Large Female Dog No. 6208. Received 500 c.c. Water Daily.

Date.	Urine.				Fæces.	Remarks.
	Volume, c.c.	Sp. Gr.	Total N, gr.	Ca gr.	Ca	
May 24	585	1010	4.82	0.035	0.533	
25	520	1010	3.88	.024		
26	560	1010	3.95	.030		
27	460	1008	3.79	.015		
28	480	1000	3.72	.015		First operation.
29	670	1006	4.19	.019		
30	370	1009	3.09	.048	1.177	Urine contains small amount of intestinal mucus fæces.
31	500	1010	4.41	.009		
June 1	390	1010	3.93	.018		
2	410	1008	3.59	.011		
3	480	1006	3.94	.013		
4	470	1008	3.81	.019	2.263	
5	440	1008	—	.018		Second operation.
6	450	1009	—	.019		
7	460	1010	—	.016		

Explanation of Table V.—At first operation four parathyroids were removed, but no signs of tetany appeared afterwards. June 5 another operation was performed, but no parathyroid could be found. On June 17 both thyroid lobes were removed and tetany appeared, showing that some interior parathyroid was extirpated together with thyroid. This shows clearly that a comparatively small amount of parathyroid tissue is able to keep up metabolic processes well enough to prevent the appearance of tetany. The composition of the urine of this animal is practically the same as in the normal control (Table IV) whereas the calcium in the fæces shows an increase.

TABLE VI.

Large Female Dog No. 1308. Put into Cage March 13. 500 c.c. Water Daily. Weight at Beginning of Experiment 19.2 kilos.

Date.	Urine.					Fæces.	
	Volume c.c.	Spec. gr.	Total N	Cl as NaCl	Ca	Ca	Remarks.
March 15	610	1021	4.86	4.10	0.053		
16	480	1020	4.72	3.2	0.024		
17	355	1022	4.88	2.2	0.018		
18	425	1021	4.28	2.6	0.016		
19	440	1020	4.84	2.7	0.021	0.127	
20	430	1019	3.69	2.6	0.014	0.003	
21	450	1018	3.15	3.1	0.016	0.153	Operation.
22	435	1018	3.94	2.9	0.023		
23	505	1013	4.28	3.2	0.030		
24	430	1020	6.73	2.9	0.025		Violent tetany.
25	330	1030	9.26	2.3	0.028		
26	350	1042	7.79	2.4	0.026		
27	340	1042	10.50	2.3	0.035		
28	170	1042	5.62	1.1	0.019		
29	140	1055	4.33	0.94	0.039	1.368	

Explanation of Table VI.—March 21, eleven distinct parathyroids are removed. March 24, tetany appears and can be noticed daily until exitus, March 29. Notice great decrease in volume of urine at onset of tetany. Specific gravity is correspondingly increased. After operation total nitrogen begins to rise and this becomes especially prominent when tetany sets in. Calcium output in urine is increased by 90 to 100 per cent. of the amount before operation. The daily output of calcium in fæces before operation 0.04 gr., after operation 0.17 gr.

TABLE VII.

Large Female Dog No. 2708. Weight 20.5 kilos. 500 c.c. Water Daily.

Date.	Urine.						Fæces.	Remarks.
	Volume c.c.	Spec. gr.	Total N.	NH_3N	NH_3 ratio.	Ca	Ca	
March 15	580	1010	3.98	0.22	5.6	0.016		
16	540	1016	3.63	0.26	7.3	0.013		
17	490	1016	4.35	0.24	5.5	0.015	1.754	
18	510	1017	6.63	0.31	4.7	0.013		
19	470	1016	3.42	0.23	6.7	0.021		
20	470	1017	3.68	0.21	5.8	0.021		Operation.
21	410	1020	4.29	0.33	7.8	0.025	0.936	
22	560	1010	3.17	0.28	9.1	0.014		
23	480	1012	4.05	0.26	6.5	0.011		
24	430	1010	3.67	0.43	11.5	0.011		
25	515	1012	3.69	2.06	55.9	0.011		
26	440	1016	3.68	1.98	53.9	0.010		
27	650	1010	3.66	1.46	40.0	0.013		
28	460	1012	3.59	1.71	47.4	0.012		
29	320	1012	2.38	1.16	48.9	0.016		
30	370	1012	3.55	1.78	50.2	0.008		
31	500	1008	3.46	1.67	48.3	0.015	2.173	Operation.
April 1	345	1020	3.26	1.43	43.8	0.016		
2	600	1012	3.31	1.62	48.8	0.092		Tetany.
3	375	1029	4.43	2.04	46.1	0.012		"
4	260	1032	4.45	1.75	39.3	0.019		"
5	620	1010	4.13	0.45	10.9	0.014		"
6	180	1036	4.96			0.015		"
7	187	1039	5.49			0.015		"
8	190	1038	5.82			0.012		"
9	185	1031	3.33			0.013		"
10	160	1032	2.67			0.011		"

Explanation of Table VII.—March 19, incomplete parathyroidectomy was performed. No tetany followed operation, but ammonia ratio was increased very much. No abnormality in other constituents of urine. March 31, complete thyroidectomy. Second day after operation violent tetany, accompanied by a great rise in the urinary calcium for one day only. Volume of urine became extremely small and specific gravity correspondingly high. Total nitrogen increased until the last two days when animal was found exhausted. Nothing conclusive can be found in the calcium excretion with the fæces. One point of special interest in this experiment is the behavior of the ammonia ratio. This latter increases rapidly after the production of parathyroid insufficiency, in spite of the fact that no clinical symptoms can be observed. After complete thyroparathyroidectomy ammonia ratio shows a tendency to fall.

CONCLUSIONS.

1. Tetany occurs spontaneously in many forms and may also be produced by the destruction of the parathyroid glands. Recent researches tend to demonstrate an intimate relation between the various forms of tetany and relative or absolute insufficiency of the parathyroid gland.

2. The parathyroid glands are independent organs with definite specific function. Whether or not this function is intimately related to that of other organs of internal secretion is not as yet proven.

3. The number and distribution of the parathyroid glands varies. Failure to produce tetany experimentally is probably due to the fact that some parathyroid tissue remains after an apparently complete extirpation. When extirpation is complete tetany appears, even in herbivora. Only a very small amount of parathyroid tissue is required to prevent this.

4. The effect of the extirpation of the parathyroid glands may be annulled by the reintroduction of an extract of these glands even from an animal of widely different character. The active principle is associated with a nucleo-proteid in the extract and may be separated with this nucleo-proteid from the remaining inert albuminous substances. Its effect in counteracting tetany appears some hours after injection and lasts several days.

5. The parathyroid glands contain no considerable amount of iodine. The parathyroid extract is not an iodine containing compound.

6. In tetany there is apparently some disturbance of the composition of the circulating fluids ordinarily prevented by the secretion of the parathyroid, which disarranges the balance of the mineral constituents of the tissues. Possibly this consists in the appearance of an injurious substance of an acid nature for such tetany may be relieved by extensive bleeding with replacement of the blood by salt solution. No actual poisonous material has, however, been demonstrated by the transference of the blood of a tetanic animal to the veins of a normal one.

7. Numerous researches have shown the important relation of the calcium salts to the excitability of the central nervous system.

Their withdrawal leaves the nerve cells in a state of hyperexcitability which can be made to disappear by supplying them with a solution of a calcium salt.

8. Tetany may be regarded as an expression of hyperexcitability of the nerve cells from some such cause.

9. The injection of a solution of a salt of calcium into the circulation of an animal in tetany promptly checks all the symptoms and restores the animal to an apparently normal condition.

10. Injections of magnesium salts probably have a similar effect but these effects are masked by the toxic action of the salt.

11. The injection of sodium or potassium salts has no such beneficial effect but rather tends to intensify the symptoms. This is true also of the alkaline salts of sodium which were studied especially in respect to their basic properties.

12. The effect of calcium is of value in human therapeutics in combating the symptoms of spontaneous forms of tetany and in relieving the symptoms in cases of operative tetany and thus tiding over the period of acute parathyroid insufficiency until remnants of parathyroid tissue can recover their function or new parathyroid tissue can be transplanted. It is in this way an important and convenient ally of the method of injecting parathyroid extract.

13. Studies of the metabolism in parathyroidectomized animals show:

1. A marked reduction in the calcium content of the tissues especially of the blood and brain, during tetany.
2. An increased output of calcium in the urine and fæces on the development of tetany.
3. An increased output of nitrogen in the urine.
4. An increased output of ammonia in the urine with

4*a*. an increased ammonia ratio in the urine.

5. An increased amount of ammonia in the blood.

Much of this affords evidence of the existence of some type of acid intoxication. Its effects are, however, not neutralized by the introduction of alkaline sodium salts and may perhaps be regarded as especially important in producing a drainage of calcium salts from the tissues which can be remedied by the reintroduction of calcium salts.

14. Emphasis must be laid upon the remarkable difference which exists between the alterations in metabolism following thyroidectomy and those following parathyroidectomy. In myxoedema there is lowered metabolism, decreased respiratory changes and lowered nitrogen output with depression of body temperature. In tetany there is increased metabolism, probably increased respiratory changes, certainly increase in nitrogen output and elevation of the temperature.

15. It is important, therefore, that in any experiments upon metabolism in relation to the thyroid and parathyroid gland, these glands should be clearly distinguished as structures exercising very different and in large part contrary effects upon metabolism.

16. In general the role of the calcium salts in connection with tetany may be conceived of as follows: These salts have a moderating influence upon the nerve cells. The parathyroid secretion in some way controls the calcium exchange in the body. It may possibly be that in the absence of the parathyroid secretion, substances arise which can combine with calcium, abstract it from the tissues and cause its excretion and that the parathyroid secretion prevents the appearance of such bodies. The mechanism of the parathyroid action is not determined, but the result, the impoverishment of the tissues with respect to calcium and the consequent development of hyperexcitability of the nerve cells, and tetany is proven. Only the restoration of calcium to the tissues can prevent this.

17. This explanation is readily applicable to spontaneous forms of tetany in which there is a drain of calcium for physiological purposes, or in which some other condition causes a drain of calcium. In such cases the parathyroid glands may be relatively insufficient.

FATAL SEPTICEMIA IN MACACUS RHESUS CAUSED BY A STREPTOCOCCUS DECOLORIZED BY GRAM'S METHOD.[1]

By R. V. LAMAR, M.D.

(From the Rockefeller Institute for Medical Research, New York.)

The interest which is being displayed in the group of pathogenic cocci which are decolorized by Gram's method of staining justifies the description of a streptococcus obtained from three monkeys (*Macacus rhesus*) which died spontaneously while in the laboratory. A further interest and importance attaches to the description of the streptococcus in view of the far greater use at the present time of monkeys in experimental laboratory work. The nature and causes of the diseases of the lower animals are only less important than of those of man, and we require particularly, in our experimental work, to understand fully the diseases of laboratory animals.

The monkeys from which the streptococcus was obtained had been previously used in an experimental study of syphilis. For several weeks before they were taken ill they were apparently in perfect health and had been returned to the house where stock animals are kept. All three died during the first few days of February, 1908—before the fifth. The first one became ill during the latter part of January and died after about ten days. The other two died much more quickly, the whole duration of illness in each being only two or three days. Of the two which died so soon after showing signs of illness one occupied the same cage with the one which was taken ill first. The third animal occupied a separate and removed cage, but it was attended by the same keeper who looked after the others.

In none of the animals was the portal of entry of the infecting streptococcus ascertained; nor were special pathological foci present in any. The visible lesions consisted of punctate hemorrhages in

[1] Received for publication October 20, 1908.

the serous membranes, congestion of the lungs, moderate enlargement of the spleen, and cloudy swelling of the viscera.

I shall describe the streptococcus fully not because its intrinsic importance may justify a detailed description of its properties but in order that future observers may be able to identify it. I have myself suffered from the very inadequate descriptions, in the literature of the subject, of what may be allied forms. It is highly improbable that the organism in question has been noted before; at least there is no description from which it may be identified. Those organisms mentioned in the literature which bear any resemblance to the streptococcus will be briefly considered after its characteristics have been set forth. Here it is sufficient to say that no previous description deals with a Gram negative streptococcus which was obtained from monkeys or which is so highly pathogenic for other animals as this one. Even when compared with *Streptococcus pyogenes* this streptococcus possesses a high degree of virulence for rabbits and white mice which it retains unimpaired after many months of cultivation upon artificial media.

DESCRIPTION OF THE MICROORGANISM.

Source.—Three cultures, designated (1), (2) and (3) and later found to be strains of the same organism, were obtained at autopsy from the heart's blood of three monkeys (*Macacus rhesus*) which had died under natural conditions. Except for less vigor of growth and less resistance to untoward influences on the part of (3), no material differences can be detected between the three strains. All statements in the description apply alike to each strain unless otherwise stated.

Morphology.—There is least irregularity in size and intensity of staining in preparations made from cultures upon glucose-sheep-serum agar kept for eighteen hours at 37°. Under such conditions the organism appears as a coccus usually arranged in pairs, sometimes in short chains of from four to eight or ten elements. Only exceptionally may a single isolated coccus be seen. In most of the short chains the arrangement in pairs is preserved, although in the long chains which are formed in liquid media (see below) it is often wanting. In stained preparations the individual elements are round,

or elliptical with the long diameter at right angles to the axis of the chain. Very often the opposed surfaces are less convex, more nearly flat, than the removed ones. There is little variation in size, the smallest measuring perhaps 0.7μ, and the largest about 1.2μ, in diameter. The average size is about 0.8μ or 0.9μ. In impression preparations made from colonies no chains are observed, but along the thin edges of the preparation the arrangement in pairs may be seen. In hanging-block preparations there are isolated pairs and short chains made up of from two to four or five pairs. Here the individual cocci are larger than in stained preparations and average about 1μ in diameter. In the hanging drop made from eighteen hour old bouillon cultures at 37° the coccus does not appear singly but in pairs or, most often, chains containing from ten to forty or more elements. Here, too, the size is larger than in stained preparations. In the water of condensation of glucose-sheep-serum agar cultures, where vigorous growth takes place, very similar appearances obtain as in bouillon except that isolated pairs are very rare and that the chains often contain hundreds of elements. These long chains are winding and they often attain a length greater than the diameter of the field of a one-twelfth objective (Oc. Leitz 3). In bouillon containing sugar there is more tendency to the formation of long chains than in plain bouillon and growth is more rapid and profuse.

No appearances suggestive of spore formation have been encountered.

Motility has been absent in preparations made from cultures fourteen, eighteen, twenty-four, thirty, and thirty-six hours old at 37°, the surface growth upon glucose-sheep-serum agar, the water of condensation, bouillon and milk being utilized for the examination. Attempts at capsule staining by the methods of Welch and of Buerger in cover-glass preparations made from the body fluids and tissues of animals killed by the organism, and from eighteen and twenty-four-hour cultures upon glucose-sheep-serum agar, upon Löffler's medium, in milk, and in Hiss's sugar serum waters, have failed to reveal the presence of a capsule.

The organism stains readily and well with dilute solutions of the aniline dyes ordinarily used for staining bacteria, and more

sharply with basic than with acid ones. It is not acid fast. It is decolorized by the method of Gram and by Nicolle's modification. In view of the occurrence, frequent in the older literature and still occasional in the newer, of such expressions as " stains well by Gram's method," " stains poorly," " almost completely decolorized," etc.; of the contradictory statements regarding the behavior of many organisms to Gram's method; of the confusion which has resulted from the use of such expressions and statements; and, finally, in view of the fact, that of the Gram negative organisms some decolorize more readily and more rapidly than others, it would seem pardonable to add that the observation is made after maximum decolorization as apparent to the unaided eye. While the streptococcus in question does not decolorize with the rapidity with which *Micrococcus intracellularis* or *Micrococcus catarrhalis* do, still in thin sections it is always decolorized in two or three minutes' treatment with alcohol, and in thinly spread cover-slip preparations within one and one-half minutes, usually within from forty to sixty seconds.

Involution Forms.—In twenty-four cultures upon glucose-sheep-serum agar at 37° there is a moderate degree of irregularity in size, most of the elements which are not of the average normal size being larger and only a relatively small number being smaller. " Arthrospore " formation is not infrequent. Sometimes a short chain is made up wholly of large forms which may reach a diameter of 1.5μ. Usually the larger forms stain more intensely than the normal ones, while the smaller forms show less avidity. After forty-eight hours' incubation abnormal forms are more numerous than after twenty-four hours but they are still far outnumbered by normal ones. There may be seen a moderate number of pairs in which one element is of normal or slightly enlarged size and well-stained, the other much smaller and less sharply stained. Even after ten or twelve days when well protected from drying the appearances are very much the same as after only forty-eight hours, there having taken place very little increase in the ratio of abnormal to normal forms. After remaining three months in the incubator, when the medium is quite dry and the growth dry, shrunken and glazed in appearance, the cocci are quite sharp in outline and stain fairly well. Preserva-

tion is remarkably good after five months in the ice-chest (5°–8°). Under each of these conditions viability has been lost.

When grown upon glucose-rabbit-serum agar, plain rabbit-blood-agar, and Löffler's medium involution occurs as above described. But in bouillon it takes place somewhat more rapidly and is manifest in a proportionately large number of individuals. Even after eighteen hours many pairs in which one element is half as large again as the other may be seen. In bouillon cultures which have been kept for a few days at 37°, there are many large round, oval and ovoid forms which may reach a size several times the average normal. Often they are situated in the middle of a chain but frequently they are seen at one or both ends, and sometimes at various points between the ends and the middle of the chain. Not infrequently a whole chain of ten or more elements is composed of cocci twice the average normal size. After eleven weeks the state of affairs is much the same.

Upon agar, plain and with glucose, involution occurs more rapidly and is more marked than in any other medium. It is extensive even after eighteen hours when the growth is still slight and has not yet reached its maximum.

In milk there is much less tendency to involution than in bouillon although in it after several weeks nearly all of the cocci are very large. Upon potato involution occurs fairly rapidly and is moderate in its extent but less marked than upon agar.

In suspensions of the growth upon glucose-sheep-serum agar in normal salt solution with toluol added the organisms are well preserved after forty-eight hours in the incubator as well as at room temperature.

Cultural Features.—The organism is a facultative anaerobe.

Stroke cultures upon glucose-sheep-serum agar; twenty-four hours, 37°: growth moderate in amount; thin, slightly raised, glistening, opalescent streak with edges made up of semi-confluent colonies. (When a very small amount of growth has been transferred in making the inoculation the colonies may remain discrete.) They are small, punctiform up to 0.5 mm., convex or pulvinate, smooth, glistening, opalescent. Chromogenesis is wanting and no odor is produced. To avoid needless repetition it may be said here

that this applies to cultures in all other media in which the organism has been grown. The growth may be scraped away with ease but it remains very finely granular and is never viscid or slimy. With the growth which has been scraped away a fine, smooth emulsion may be made, but unless it be judiciously and thoroughly well rubbed up in the beginning with only a trace of moisture the emulsion is prone to be uneven and to contain small clumps. In cultures forty-eight hours old of which there has been some drying this tendency to cohesion and to the formation of clumps is very difficult to overcome.

Clouding of the medium, presumably due to precipitation of the contained albumin by the acid set free in the fermentation of the glucose, is present to a slight degree. It may indeed be noticed in cultures only eighteen hours old. The cloudiness steadily increases until within the course of a few days the medium is opaque and white throughout. In the water of condensation growth is profuse. A coarsely granular grayish-white precipitate is formed. At first it is evenly distributed throughout, sometimes forming a thin pellicle, later it has fallen to the bottom leaving the fluid above clear. Upon microscopical examination a considerable proportion of this precipitate is seen to be made up of coagulated albumin. The water of condensation is coagulated in a jelly-like mass quite late, usually after some ten or fifteen days.

When rabbit serum is substituted for the sheep serum growth is the same, sometimes slightly more profuse, but a very striking difference is noted in the appearance of the medium. The cloudiness above referred to does not appear until after four or five days; and then is very slight in degree, and is limited to the immediate proximity of the growth, while the sheep serum medium under the same conditions is white and opaque throughout. This is what occurs when the glucose agar before the addition of the rabbit serum is faintly alkaline to phenophtalein. When the reaction is acid the phenomenon appears sooner and increases in intensity with the degree of acidity as well as with the age of culture; but it never approaches in intensity that which occurs in the presence of sheep serum no matter how acid in reaction the medium may be or how old the cultures.

Upon sheep-serum agar without glucose the growth is similar to that described above but is not so profuse and is more delicate in appearance, the edges being slightly raised above the level of the middle portion of the streak.

Upon plain agar with rabbit's blood growth is like that upon glucose-sheep-serum agar except that it is more nearly transparent and that there is less precipitate in the water of condensation. "Hemolysis" does not occur.

With Löffler's medium and with solidified egg to which veal broth has been added growth occurs as upon glucose-sheep-serum agar. It is more difficult to remove.

Upon plain agar and upon glucose agar growth is moderate in amount but decidedly less than upon any of the media considered above. The streak is narrow and flat, the edges being slightly higher than the interior, and made up of punctiform, glistening, translucent, semi-confluent colonies. The medium remains unchanged. There is a small quantity of a finely granular white precipitate at the bottom of the water of condensation. Upon shaking it diffuses evenly. The maximum amount of growth is reached before thirty-six hours; indeed there is very little increase after twenty-four hours.

Upon potato growth is moderate in amount. It is invisible to the unaided eye. When the surface is scraped the growth comes away readily in small, white, rather dry, finely granular masses. The medium is unchanged.

In agar stab cultures growth is slight and most marked along the track of the wire where it appears as a fine, grayish-white, filiform streak, sometimes beaded at the bottom. Surface growth is very scant and is confined to the immediate edges of the incision.

Gelatin stab cultures 20°–22°: Growth is slight and slow, appearing after from thirty-six to forty-eight hours as a fine grayish white, filiform track, beaded for the lower two-thirds of its extent. Surface growth as in agar stab cultures. The amount of growth very slowly increases for five or six days until it may be said to be moderate. Liquefaction does not occur. Also gelatin cultures kept continuously at 37° for six weeks suffer no impairment of their power to solidify when placed in the ice-chest.

Bouillon: twenty-four hours at 37°: surface free; slight uniform cloudiness. On shaking a small amount of finely granular whitish sediment arises and diffuses evenly. The reaction has become faintly acid. Acidity increases slightly as growth advances. The amount of sediment slowly increases for three or four days, after which it remains stationary. During this time the liquid has gradually become clear, all growth having settled to the bottom. In bouillon containing one per cent. of glucose, maltose, or lactose, much more growth occurs than in plain bouillon. This is especially true of glucose broth in which there is profuse growth. The precipitate is coarser than in plain bouillon and acid production is very much more rapid and pronounced. The addition of sheep serum to bouillon favors growth. Glycerin bouillon proved the poorest of the media employed for the organism. In litmus milk, which is a favorable medium, slight acid reaction may often be detected within eighteen hours. This increases until the maximum intensity of pink color is reached in from five to seven days. Beginning coagulation is indicated at the end of two weeks or during the third week. It progresses very slowly requiring several days for its completion. The coagulum is pink and made up of large coarse flakes. The whey is clear and colorless. The reaction remains acid. Peptonization does not occur.

Gelatin plate cultures 20°–22°: colonies are just visible to the naked eye upon the third day. Under the low power they appear round or oval, grayish translucent, and much lighter and somewhat more coarsely granular than upon glucose-sheep-serum agar. The edges are fairly sharp but irregular in outline. Subsequently the colonies increase slightly in size but they never exceed a diameter of 0.5 mm.

Glucose-sheep-serum agar plate-cultures at 37°: colonies may be seen at the end of twelve hours. They increase rapidly in size up to twenty-four hours; slightly from twenty-four to thirty-six; none after thirty-six. At twenty-four hours: superficial colonies are round, varying in diameter from 0.3 to 0.8 mm., convex or pulvinate, smooth, glistening, nacreous. Under the low power they are brown and finely granular without a nucleus but with a narrow circumferential zone which is of a lighter brown color than

the interior. (Occasionally after forty-eight hours a colony may show a nucleus.) The edge is almost entire and very thin. There is slight whitening of the medium in the immediate vicinity. It is seldom that a colony attains a diameter of 1 mm., although upon very moist plates containing only three or four colonies a diameter of 1.5 mm. may sometimes be attained.

The deep colonies appear in two forms according as they are situated within the substance of the medium or upon its lower surface against the glass. Those in contact with the glass are very similar to the superficial ones except that they are flat, of a lighter grayish color, and more nearly translucent. The others are smaller than the superficial ones, either round or elliptical, whiter by reflected light, more coarsely granular and of a darker brown color by transmitted light. The elliptical ones are usually sharply defined at the poles but poorly so along the sides. None show the narrow light, circumferential zone of the superficial colonies.

Upon plain and glucose agar plates the colonies are as above described, only smaller with edges sharper in outline and very finely serrated.

The most favorable solid medium for the streptococcus is glucose-sheep-serum agar. One-half per cent. glucose favors growth more than one per cent., 1.5 per cent., or two per cent. Of the liquid media milk and one per cent. glucose, plus 0.5 bouillon (before sterilization) are alike the best suited for its growth.

Physical and Biochemical Features.—In fermentation tubes containing bouillon (originally sugar free) plus one per cent. of glucose, lactose, maltose, saccharose, glycerin and mannite gas formation does not occur. Acid is produced, however, in those tubes containing glucose, saccharose, lactose and maltose, but not in those containing glycerin and mannite. The acidity is greatest in the presence of dextrose, yet marked in broths containing the disaccharids. Growth occurs in both arms of the tubes.

By the use of Hiss's sugar serum waters the fermentative abilities of the organism are found to be slight in degree and range. With dextrose and levulose acid production is noticed in twenty-four hours; but coagulation does not occur until between the sixth and tenth days. The attack upon the disaccharids is very weak.

Lactose water acquires a sharp color only after several days and solidification does not occur until after three weeks. Sometimes maltose water is coagulated after two or three weeks; sometimes not at all. But it is always slowly reddened. The same is true with saccharose. Of the polysaccharids, starch and dextrin waters are turned pink very slowly and never coagulated; while inulin is not attacked at all. Nor are glycerin or mannite split.

Indol production occurs but very slowly and the reaction obtained is slight.

Influence of Reaction upon Growth.—Growth occurs in media having before sterilization a reaction slightly alkaline to phenolphthalein; also at a reaction of + 1.5; and at intermediate points. The optimum reaction is + 0.5.

Vitality.—The organism preserves its vitality for a long time upon artificial culture media. Longevity is greatest in glucose-sheep-serum agar cultures kept in the ice-chest at a temperature of from 5° to 8°. After three months under such conditions vigorous growth occurs in the transplantations. At room temperature, 22° to 27°, viability does not persist for so long a time. When carefully protected from drying cultures upon glucose-sheep-serum agar, plain agar, and in bouillon often remain alive, for from six to eight weeks; almost always for four weeks. In the incubator vitality seems to be preserved longest in milk, most cultures giving growth after four weeks although sometimes growth has ceased to occur in the transplantations during the third week. In bouillon the average length of time during which vitality is preserved is about three weeks. Upon glucose-sheep-serum agar it is still less—about two weeks, often not more than ten or twelve days.

The relation which the reaction of the medium bears to the life of the organism is well shown in a number of vitality experiments. These were made in a parallel manner with specimens of bouillon slightly alkaline to phenophthalein and having a reaction of + 0.5. One may be quoted.

April 7, 1908. Transplantations upon glucose-sheep-serum agar were made from one culture of each strain in plain slightly alkaline bouillon and in plain +0.5 bouillon. All cultures were eleven days old at 37°. In each instance one maximum loop full of the bouillon

cultures was transferred. After twenty-four hours incubation at 37°:

Transplantations from Slightly Alkaline Bouillon.	Transplantations from 0.5 Bouillon.
Strain (1) shows one colony.	(1) Considerable growth of confluent colonies.
Strain (2) shows two colonies.	(2) Slightly less growth.
Strain (3) shows none.	(3) Slight growth, many colonies.

Temperature Relations: Growth does not take place at temperatures below 17°, or at or above 42°. The optimum temperature is 37°. Upon glucose-sheep-serum agar (the most favorable solid medium):

At 17.5°, growth is extremely slight and is visible only after four days.

A 20°–21°, it is scant; visible afer 48 hours.

At 25°–27°, it is moderate; barely visible after 18 hours.

At temperatures between 30° and 40°, growth is more marked and may be seen after twelve hours.

The organism does not survive an exposure to a temperature of 80° prolonged for fifteen minutes. There was often a considerable difference in the resistance to heat offered by various bouillon cultures of the same age; and cultures of strain (3) were invariably killed by less heat than those of (1) and (2). All, however, resisted a temperature of 65° for fifteen minutes. The relation to very low temperature has been referred to.

Cultures upon solid media in capped tubes remain viable about twice as long as those in uncapped ones.

No proteolytic action is exerted and lipase is not produced.

Toxin Production.—Several flasks of bouillon kept continuously in the incubator were inoculated with strains (1) and (2). They were re-inoculated every three or four days for two or three weeks after which length of time the contents were filtered. The filtrates in amounts of from two to eight cubic centimeters produced no visible effects when injected intravenously into rabbits which are extremely susceptible to infection with the living organism.

Hemolysis has not been observed *in vivo*. Of many bouillon cultures which were tested *in vitro* for hemolysin only a few produced hemolysis and then only when large amounts were used. The corpuscles of the rabbit, guinea-pig and hen were employed.

Pathogenicity.—Belgian hares and white rabbits are alike very susceptible to artificial infection with the streptococcus and, as a rule, die quickly as a result of it. In white mice also the organism produces septicemia and death, but proportionately larger doses are required. White and hybrid rats, especially old ones, are very refractory but in them death sometimes follows intra-abdominal and intracardiac injections of large amounts of cultures. Subcutaneous injections of twenty times an amount fatal for a rabbit weighing 1800 gr. do not produce death, but are followed by some loss in weight, and sometimes by a slight indurated swelling at the site of injection. The swelling slowly subsides and health is regained. Subcutaneous, intra-abdominal and intra-cardiac injections of cultures into guinea-pigs are followed inconstantly by only a slight loss of weight which is quickly regained; except when excessively large amounts are introduced directly into the circulation when the loss of weight is more pronounced and persists longer, and when there are some evidences in the behavior of the animals of illness. But death does not follow even intracardiac injections of these large amounts. In the pigeon injections of large amounts of cultures into the pectoral muscles produce a more or less pronounced local reaction—redness, swelling, induration—which persists for several days and only slowly subsides. Abscess formation has not been observed. Appearing a few hours after the injection there is a slight general disturbance manifested by reluctancy to move, apparent inability to maintain a firm hold of perch and consequently falling off when placed upon it, refusal of food, and finally, by a slight loss of weight. These manifestations disappear quickly and health is completely regained. The hen responds in a similar manner.

Mice.—Subcutaneous injections in white mice are followed by death within from forty-eight to seventy-two hours, usually after about sixty hours, except when a minimal fatal amount (about 0.1 or 0.2 c.c. of a twenty-four hour bouillon culture) is injected. Then death is sometimes delayed until four or five days after the injection. At the site of injection is a rather dry, plastic, fibrinous exudate moderate in amount, and a necrosis of the surrounding subcutaneous tissue often extending into and involving the con-

tiguous muscles. Abscess formation has never been observed. Usually the spleen is slightly enlarged, soft and moist. In one mouse hemorrhagic infarction occurred in the spleen. The lungs are always of an intensely bright scarlet color as soon as exposed; they crepitate throughout. Microscopically the congestion is seen to be marked and many hemorrhages into the alveoli to have occurred. In the dead mice the streptococcus is always readily found pure in smears and by culture from the local exudate, the heart's blood, lungs and spleen. In the exudate the cocci are very numerous and appear mainly in isolated pairs, although occasionally a very short chain of three or four pairs or a small clump is observed. There are few polymorphonuclear leucocytes in the exudate, and only exceptionally is phagocytosis seen. In the heart's blood the organisms are fairly numerous, and usually grouped in short chains containing ten or fifteen elements although a few isolated pairs may be seen. In the lungs they are present in large numbers within the blood vessels and mixed with the blood which has escaped into the alveoli. Likewise large numbers are found in the spleen. Cultivation from each of these sources succeeds easily, the resulting colonies tending to remain discrete.

Intra-abdominal injections produce a general peritonitis with a small amount of serofibrinous exudate. Septicemia occurs and death follows in two or three days.

Rabbits.—Belgian hares and white rabbits are both very susceptible to infection with the streptococcus. For them the organism possesses a high degree of pathogenicity—higher than for any other animal upon which it has been tested. After subcutaneous injection septicemia develops and death occurs in about three days. The local reaction is like that which occurs in mice. Intra-abdominal and intravenous injections alike produce death with great rapidity when the amount of culture injected is moderate or small. The minimal fatal dose for rabbits weighing from 1,700 grams to 2,000 grams has been approximately about 0.2 c.c. or 0.3 c.c. of a twenty-four hour bouillon culture; or less than one fiftieth of a very small loop (made of No. 24 platinum wire and having an inner diameter of 2.5 mm.) of a twenty-four hour growth upon glucose-sheep-serum agar. When such small amounts have been injected intra-

abdominally in some rabbits and intravenously in others, death has often followed more quickly after the intra-abdominal injection and sometimes it has seemed as though a smaller amount was fatal. Death is usually delayed after a *very* small dose for five or six days. With small doses of 0.4 c.c. or 0.5 c.c. of a twenty-four hour bouillon culture, or a fortieth to a thirtieth of such a loop as mentioned above of a twenty-four hour growth upon glucose-sheep-serum agar, death usually occurs within from forty-eight to seventy-two hours, while when larger amounts are injected death occurs before the lapse of twenty-four hours, often before eighteen.

Following an intra-abdominal injection there is a general peritonitis with a hemorrhagic sero-fibrinous exudate. As a whole the peritoneal surfaces are very red, moist, dull and lusterless, with here and there fibrinous deposits. Frequently the fibrin has been greatest in amount about the spleen. There is a small amount— 5 to 10 c.c. —of rather thick, turbid, yellowish-red fluid containing smaller and larger flocculi of fibrin. Microscopically the fluid exudate contains only a moderate number of pus cells and a great many cocci. The common arrangement is in isolated pairs and small groups of pairs although chains of four or five elements may be seen. The position is almost always intra-cellular, phagocytosis being very rare and when present slight in degree. The changes in the viscera are like those to be described as following intravenous injection except that the spleen is larger, softer and more moist.

When death has resulted from an intravenous injection the most striking changes are afforded by the lungs and kidneys. The lungs are intensely congested and their appearance at once suggests minute hemorrhages. Sometimes there is marked edema and the frothy fluid is blood stained. The spleen and liver are engorged. The kidneys are enlarged, dark in color, and show throughout numerous fine hemorrhages about 1 mm. in size. The cortex is increased in thickness and the pyramids are livid. There is cloudy swelling of the epithelial cells, not only of the convoluted but often also of the collecting tubules, and sometimes coagulative necrosis is observed. The urine contains (and this holds true after intra-abdominal injections also) a moderate amount of albumin, usually a moderate number, sometimes many, casts, a few blood and epi-

thelial cells and many organisms which upon cultivation are found to be the coccus unassociated with other bacteria. Most of the casts are coarsely granular, a few hyaline and a few epithelial. Often after an intravenous injection hemorrhagic transudations have occurred into the serous cavities and the streptococcus may be cultivated from the fluid. Attempts at cultivation from the spleen, liver, kidneys, lungs, heart's blood and urine succeed easily. Likewise the coccus is found in smears from these sources. Attempts to recover the organism from the bile have been uniformly unsuccessful.

In microscopical sections of the organs the cocci are readily seen. They decolorize by the Gram-Weigert method. Pairs and short chains occur. For the most they are situated within the blood vessels although in the lungs many may be seen with the extravasated blood in the alveoli. Also they may be found within the parenchyma of the kidney and often free in the lumina of the tubules.

Scratches were made upon the ear in two rabbits and a small amount of culture rubbed in. An erysipelatous condition confined to the ear occurred in both. In one animal it was very severe. The reaction subsided in ten or twelve days and was followed by desquamation of the skin and the loss of considerable hair. When the inflammation was at its height the animals appeared to be ill.

Immunity and Serum Reactions.—Rabbits were immunized by intravenous injections of pure cultures. For the first injection bouillon cultures were killed by being exposed to a temperature of 80° for fifteen minutes. Then live organisms were employed, at first heated to 60° for fifteen minutes and in very small quantities, later unheated and in larger amounts until in one instance at least fifty times an otherwise certainly fatal amount was injected. This plan was adopted because it overcame the very high degree of virulence of the organism for the rabbit, and because it at the same time afforded a serum of a fairly high agglutination titer.

By means of agglutination tests with the serum of such an immune rabbit the streptococcus was easily differentiated from the streptococcus of "lumps" in guinea-pigs, five strains of *Streptococcus pyogenes,* two of *Micrococcus intracellularis,* and from three

unclassified Gram negative cocci obtained at autopsies upon horses suspected of having meningitis. The serum of one animal (Rabbit No. 23) immunized with strain (1) agglutinates that strain, as well as strains (2) and (3) at a dilution of 1 to 250. The same serum in a dilution of 1 to 20 does not agglutinate any other of the organisms named above except *Streptococcus pyogenes* which is slightly agglutinated at this strength. At a dilution of 1 to 40, however, there is no trace of agglutination of *Streptococcus pyogenes.*

No bactericidal effect of the immune serum was detected.

Until the present time (October 20), after having been maintained upon artificial culture media for eight months, none of the three strains of the streptococcus has suffered any appreciable loss of virulence for white mice or rabbits. This holds true for cultures kept most of the time at room temperature as well as for those preserved in the ice-chest.

Habitat.—Concerning the habitat of the streptococcus nothing has been found out. Several attempts to find it in the mouths and throats, axillæ and groins, and about the anus, of normal monkeys have failed. In autopsies upon four monkeys which died while the study was in progress it was not encountered.

In reviewing those organisms, mentioned in the literature, which might be considered as more or less similar to the streptococcus above described one is confronted by meager notes, often of a single observation only, and frequently by contradictory statements concerning important characteristics, so that in many instances it is impossible to gather even a fair idea as to the nature of the organism mentioned. Such observations are almost useless for comparative study and will be only cited and not dwelt upon. But there are a few organisms which have been studied more closely and of which there exist better descriptions, although concerning certain of these contradictory statements are made. The latter group will be considered first.

In the present state of our knowledge the organism most like the streptococcus just described is the streptococcus of "lumps," a chronic infectious lymphadenitis of the guinea-pig. It was described by Boxmeyer (1) in 1907 and said to stain by Gram's

method. Before the date of Boxmeyer's paper Dr. Flexner (personal communication) had observed a coccus associated with a disease in guinea-pigs apparently identical with "lumps." He noted, however, that the coccus decolorized by Gram's method. I have made observations upon seventeen strains of this organism and have found it to be quite similar to the one described by Boxmeyer, except mainly in regard to its behavior to Gram's method and in regard to the nature of the growth upon solid media. Stroke cultures upon solid media almost always appear as a raised, glistening, vitreous slimy and viscid streak which after twenty-four to forty-eight hours has dried into a thin, flat, grayish-white film. In addition to well-defined cultural differences this streptococcus may be differentiaed from the one obtained from the monkeys by agglutination tests (see above).

Perhaps as next in importance from the standpoint of similarity should be considered the "Drusestreptococcus" or *Streptococcus equi,* which is the cause of "strangles," "Druse," or "gourme" in horses. The disease is characterized by a chronic inflammation of the mucous membrane of the nose and throat usually complicated by abscess formation in the anatomically related lymph nodes. Occasionally pyemia occurs with multiple abscesses in the liver, spleen and lungs. The streptococcus was described almost at the same time but independently by Schütz (2), Sand and Jensen (3) and Poels (4). Only Sand and Jensen made mention of the behavior to Gram's method and they stated that the organism retains the stain. Of later investigators all except Rabe (according to von Lingelsheim) seem to agree that it stains by Gram's method, although Bongert (5) writes:—"The decolorization in alcohol must not extend too long as otherwise the Drusestreptococcus gives up the stain." He has seen complete decolorization after forty-five seconds' treatment with alcohol. According to him growth occurs in milk without causing a change of reaction or coagulation, while Nocard and Leclainche (6) state that milk is coagulated in from twenty-four to forty-eight hours. Except for this difference there is a close agreement as to the characteristics of the organism. In liquid media it forms very long chains. Perhaps its most striking single culture feature is the appearance of stroke

cultures upon agar and solidified blood serum. The streak is at first raised, glistening, vitreous, mucoid. After three or four days it has become a thin, dry, shrunken, iridescent deposit. Sometimes superficial, well isolated colonies upon agar attain a diameter of three or four millimeters. White mice die within three or four days after subcutaneous inoculations of cultures. Guinea-pigs and rabbits are refractory, subcutaneous injections having no effect, or, at the most, producing only a slight local reaction in the rabbit. Intravenous and intra-abdominal injections of very large amounts, however, produce death in both. As to just what relationship may exist between the Drusestreptococcus, the streptococcus of " lumps," and the streptococcus described above further work is necessary for a determination.[2]

Closely related to, and perhaps identical with, *Streptococcus equi* is the organism which Schütz (7) found associated with pneumonia in the horse, and which has been called " Brustseuchecoccus." Apparently it has not been as much studied as *Streptococcus equi;* and concerning many of its characteristics there are wide diversities of opinion. In his original communication Schütz states that the organism is a " small, capsulated, oval bacterium." It decolorized by Gram's method, and to this fact he attached diagnostic significance. Rust (8) confirmed the statement of Schütz as regards morphology and staining reactions. Later writers refer to the organism as a coccus and do not consider it capsulated. Lignières (9) holds that it is a streptococcus, that it stains by Gram's method (as Schütz is said to have later admitted) and that it is identical with *Streptococcus equi.* Bongert (10), on the other hand, does not agree with Lignières concerning the identity of the two organisms. Until more work has been done it can not be said just what the " Brustseuchecoccus " is.

[2] Since the paper was written I have had an opportunity of obtaining six strains of *Streptococcus equi* from horses having " strangles." All decolorize by Gram's method. Without discussing at length the characteristics of the three Gram negative streptococci, streptococcus obtained from monkeys, the streptococcus of " lumps," and *Streptococcus equi,* it may be stated that a comparative study reveals close similarity between the streptococcus of " lumps " and *Streptococcus equi* and well-marked differences between these and the streptococcus obtained from monkeys which are sufficient to establish the streptococcus obtained from monkeys as a distinct organism.

Ostertag (11) has described as the cause of the infectious aborting of mares a streptococcus which decolorizes by Gram's method. It occurs in short chains. Cultivation upon artificial media is very difficult and growth is less active with each succeeding transplantation. Upon serum agar a very delicate, scarcely visible streak is formed. Growth does not occur in gelatine or milk. For mice, guinea-pigs and rabbits the organism is not pathogenic. These facts serve to differentiate it from the streptococcus obtained from the monkeys.

The same is true of the streptococcus which Hecker and Ostertag (2) have described as the cause of a specific vaginitis in cows. It, too, decolorizes by Gram's method but is pathogenic for cows alone.

In 1884 Nocard and Mollereau (13) observed in the milk of cows affected with a chronic contagious mammitis a streptococcus which they believe to be the cause of the disease. Hess and Borgeaux (14) in 1888 described a streptococcus which they considered the cause of the same disease (mammite streptococcique de la vache; gelber Galt). Later the two organisms have been shown to be identical. In regard to the behavior of the streptococcus to Gram's method Nocard and Mollereau wrote originally that if the action of the alcohol be prolonged it decolorizes. Later Nocard and Leclainche (15) stated that it "stains poorly by Gram's method." Recently Steiger (16) in a study of a series of cases of chronic mammitis in the cow, apparently the same disease, gives a description of a streptococcus which tallies with that of other observers except for the statement that it stains by Gram's method. The organism described by Nocard and Mollereau coagulates milk solidly within from twenty-four to forty-eight hours. Introduced directly into the udder of a cow or goat a culture produces a mammitis indistinguishable from that arising naturally. For other animals, no matter how introduced, it is not pathogenic. Klein's (17) "*Streptococcus radiatus*" found in a serofibrinous exudate from the udder of a cow stained by Gram's method. He considers it a different organism from that of Nocard and Mollereau.

Passing from this group of streptococci which are fairly well characterized we may turn our attention to the notes concerning

other Gram negative organisms about which less is known. Even in the imperfect reports concerning these enough is stated to make it seem highly improbable that any one of them is closely related to the streptococcus obtained from the monkeys. Only those features in which they differ widely from the organism under consideration need be mentioned.

Galtier (18) in 1891 described as the cause of "courade"— a wide-spread epidemic infection of newly-born calves, lambs, kids and pigs—a motile, Gram negative coccus. It grew upon artificial media at from 12° to 25°. Gelatin was liquefied by it.

In preparations made from the throat of a patient having pseudo-membranous angina, Étienne (19) found, and grew in pure cultures, a streptococcus which decolorized with great rapidity by Gram's method. Ziemke (20) in a footnote to an abstract of Étienne's communication mentions a streptococcus which he obtained from the blood of a hog which had died of septicemia. It was very similar to the one described by Étienne, constantly decolorizing by Gram's method. Inasmuch as the notes are scant and the observations have not been confirmed it is useless to speculate as to the real nature of the organisms.

Cottet and Tissier (21) in 1900 described a streptococcus of which the prinicipal characteristic was that it decolorized by Gram's method. It was found in the urine of a patient having a purulent cystitis and in the fæces of a nursing infant which had diarrhœa. In diameter it was scarcely more than 0.5μ. Colonies upon agar were fine and almost transparent; after twenty-four hours, they did not exceed 0.4 to 0.5 mm. in diameter. The organism was killed by an exposure to a temperature of 60° for fifteen minutes.

Baruchello (22), 1905, found in the fæces of almost all normal horses and asses a Gram negative saprophytic streptococcus. He considered it different from *Streptococcus equi, Streptococcus pyogenes* and Schütz's Brustseuchecoccus.

In a study of mumps, Laveran and Catrin (23), 1893, found a diplococcus which they said decolorized by Gram's method. Later Mecray and Walsh (24) found an organism which they regarded as identical with the one observed by Laveran and Catrin, and

which they believed was only "*Staphylococcus epidermidis albus.*"

Crajkowski (25) in 1895 reported the finding of a Gram negative coccus in the blood (obtained from the ear) of patients having scarlatina. Large quantities of cultures injected intravenously into rabbits produced no effect.

In the descriptions of the following streptococci there is nothing to warrant their being considered as other than *Streptococcus pyogenes* except for the statements that the first one decolorized by Gram's method and that the second one "almost decolorized:" Doleris and Bourges (26), pus of a pelvic abscess; Barbier (27), membranous angina; Lucet's (28) *Streptococcus pyogenes bovis*; Moore (29), suppurative cellulitis in the cow; Kutschera (30), multiple abscesses in the white mouse.

It is impossible to classify the Gram negative coccus found by Lesage (31) in the nasal and laryngeal secretions of patients having measles. There is no mention of its cultural characteristics except that it formed zoogleæ.

The diplococcus found by van Harrevelt (32) in the flesh of a horse which had been shot on acount of an enteritis decolorized by Gram's method. It did not form chains. Gelatine was liquefied by it.

Babes's statement (33), 1889, concerning a capsulated, Gram negative diplococcus found in the blood of cows suffering with an infectious hemoglobinuria is so obscure that it may not be known with what organism he was dealing.

In the communication of Eberth (34) (mycosis in the guinea-pig, 1885), and of Penberthy (35) (valvular vegetation in a horse, 1893) no cultural features are stated. Apparently only microscopical examinations were made.

Finally, many other organisms have been found associated with various infections occurring in laboratory animals. Such are those described by Smith (36), Binaghi (37), Catterina (38), Klotz (39), and others. But it is unnecesary to discuss these here inasmuch as each one possessed some distinct feature or features, such as capsule, flagella, etc., which serve to differentiate them at once from the streptococcus obtained from the monkeys.

SUMMARY AND CONCLUSION.

A Gram-negative streptococcus is associated with one form of fatal septicemia arising naturally in *Macacus rhesus* confined in laboratories.

This streptococcus differs from *Streptococcus pyogenes* in its behavior to Gram's method of staining; in its slight fermentative powers; in the persistence of vitality for a very long time in its cultures; in its high degree of pathogenicity for certain lower animals; and in the preservation of its virulence undiminished for many months when cultivated upon artificial media.

In the present state of our knowledge this streptococcus must be considered as not identical with any streptococcus heretofore described.

I desire to express my thanks to Dr. Flexner for the opportunity of making the study and for supervision of it, and to Drs. Jobling and Noguchi for many suggestions.

BIBLIOGRAPHY.

1. Boxmeyer, *Jour. of Infectious Diseases,* 1907, iv, 657.
2. Schütz, *Zeit. f. Hygiene,* 1888, iii, 427.
3. Sand and Jensen, *Deut. Zeit. f. Thiermed. u. vergl. Path.,* 1888, xiii, 437.
4. Poels, *Fortschr. der Med.,* 1888, vi, 4.
5. Bongert, Kolle-Wassermann Handbuch, 1903, iii, 734.
6. Nocard and Leclainche, Les maladies microbiennes des Animaux, 1903, ii, 279.
7. Schütz, *Archiv f. Thierheilkunde,* 1887, xiii, 27.
8. Rust, *ibid.,* 283.
9. Lignières, Ref., *Baumgarten's Jahresbericht,* 1897, xiii, 697.
10. Bongert, *op. cit.,* 739.
11. Ostertag, Kolle-Wassermann Handbuch, 1903, iii, 835.
12. Hecker and Ostertag, Nocard et Leclainche, Les maladies microbiennes des animaux, 1903, ii, 323; and Kolle-Wassermann Handbuch, 1903, iii, 840.
13. Nocard and Mollereau, *Annal. de l'Inst. Pasteur,* 1887, i, 107.
14. Hess and Borgeaux, Ref., *Baumgarten's Jahresbericht,* 1888, iv, 33.
15. Nocard and Leclainche, *op. cit.,* 318.
16. Steiger, *Centralbl. f. Bakt., Abt. I, Orig.,* 1904, xxxv, 468.
17. Klein, *ibid.,* 1900, xxviii, 417.
18. Galtier, Ref., *Baumgarten's Jahresbericht,* 1891, vii, 57.
19. Étienne, *Archives de méd. expér.,* 1895, vii, 503.
20. Ziemke, *Baumgarten's Jahresbericht,* 1895, xi, 26.
21. Cottet and Tissier, *Comp. rend. de la Soc. de Biol.,* 1900, lii, 627.
22. Baruchello, *Centralbl. f. Bakt., Abt. I, Orig.,* 1905, xxxix, 569.
23. Leveran and Catrin, *Comp. rend. de la Soc. de Biol.,* 1893, xlv, 95, 528.

24. Mecray and Walsh, *Medical Record,* 1896, I, 440.
25. Crajkowski, *Centralbl. f. Bakt., Abt. I,* 1895, xviii, 116.
26. Dóleris and Bourges, *Comp. rend. de la Soc. de Biol.,* 1893, xlv, 1051.
27. Barbier, *Archives de méd. expér.,* 1892, iv, 827.
28. Lucet, *Annal. de l'Inst. Pasteur,* 1893, vii, 325.
29. Moore, Ref., *Baumgarten's Jahresbericht,* 1898, xiv, 46.
30. Kutschera, *Centralbl. f. Bakt., Abt. I, Orig.,* 1908, xlvi, 671.
31. Lesage, *Comp. rend. de la Soc. de Biol.,* 1900, lii, 203.
32. van Harrevelt, *Centralbl. f. Bakt., Abt. I.,* 1899, xxvi, 121.
33. Babes, *Virchow's Archiv,* 1889, cxv, 81.
33. Eberth, *ibid.,* 1885, c, 15.
35. Penberthy, Ref., *Baumgarten's Jahresbericht,* 1893, ix, 108.
36. Smith, *Jour. of Comp. Med.,* 1887, viii, 24.
37. Binaghi, *Centralbl. f. Bakt., Abt. I,* 1897, xxii, 273.
38. Catterina, *ibid., Orig.,* 1903, xxxiv, 108.
39. Klotz, *Jour. of Med. Research,* 1904, xi, 493.

AUTO- AND ISOTRANSPLANTATION, IN DOGS, OF THE PARATHYROID GLANDULES.[1]

By W. S. HALSTED, M.D., Baltimore.

PLATES II AND III.

As regards the preservation of form merely, transplantation of the parathyroid glandules has been successfully undertaken by several investigators. In combination with the thyroid gland, and before the remarkable discovery by Gley and by Vassale and Generali of a vital function for the parathyroid glandules, v. Eiselsberg, in 1892, in experiments which have become classic, made transplantations of these bodies which were not only successful morphologically but also from the functional point of view. The correct interpretation of his results, of the tetany which followed the extirpation of the transplanted thyroid gland, could not even be surmised until about four years later. Enderlen in 1898 and Payr in 1906 transplanted, with morphological success, the "epithelial bodies" as they happened to be included in the thyroid grafts. Transplantation of the isolated parathyroid glandules were undertaken by Camus as early as 1904 (Leischner) and Christiani reports in 1905 that the transplanted epithelial bodies of a cat had undergone little change in five years. The first to obtain functional proof by their transplantation was Leischner.[2] His experiments were performed on rats, the donor of the glandules being the donee. He used about eighty rats in the investigation and reports eight cases in which functional proof seems to have been obtained. My work on transplantation of the parathyroid glands in dogs was begun in the autumn of 1906, and has been continued, interruptedly, until the present time. It was undertaken with a view to determine the course to be pursued by the surgeon when a parathyroid gland has been accidentally removed or deprived of its blood

[1] Received for publication November 9, 1908.

[2] Leischner, *Archiv für klin. Chir.*, 1907, lxxxiv, 208.

supply, and in the hope that it might be attended with such success as to justify the attempt to transplant this glandule from man to man. In the course of the work many questions have arisen, and we find ourselves still hardly beyond the threshold of the investigation. In a patient suffering greatly from subtetanic hypoparathyroidism as the result of two operations by myself upon a large colloid goitre which produced distress from pressure, tetany has for three years been averted and the status parathyreoprivus made endurable by the feeding of parathyroids, by hypodermic injections of the nucleoproteids of the parathyroid gland (Beebe) and for almost one year by the administration of calcium lactate (MacCallum and Voegtlin).[3]

My first transplantations in dogs of the parathyroid glands,[4] and the first of which I know, were made into the thyroid gland of the donor; next into the spleen, on the announcement by Payr of the successful transplantations of the thyroid gland into this organ; and finally (1907–1908), on the appearance of Leischner's contribution (von Eiselsberg's clinic), within and behind the musculus rectus abdominis, and in the midline, præperitoneally.

Pfeiffer, Hermann, and Mayer[5] have made two successful autotransplantations in puppies.

Biedl, commenting on the unsuccessful attempts of Foges, Kreidl, and himself to transplant ovaries, testicles, and suprarenal glands, contrasts these failures with his success in transplantation of the parathyroid glands. He states that a year prior to his report,[6] he transplanted in two dogs into the spleen "foreign" parathyroids, and "after a time" removed both thyroid lobes as well as the parathyroids. One animal lived seven months without a trace of tetany and finally died of what seemed to be "cachexia thyreo-

[3] Reference has been made to this case in previous publications. In the two operations only one parathyroid gland was removed (specimens examined by Dr. MacCallum and myself), and this little body was recognized the instant the final resection-cut into the right thyroid lobe was made. It was not until six months later, in the autumn of 1906, that it occurred to me to attempt the transplantation of the parathyroids.

[4] Hypoparathyreosis, Status Parathyreoprivus and Transplantation of the Parathyroid Glands, *Amer. Jour. of Med. Sciences,* 1907, cxxxiv, 1.

[5] *Mitteilungen aus den Grenzgeb. der Med. und Chir.,* 1907, xviii, 377.

[6] *Wien. klin. Woch.,* 1908, xxi, 304.

priva." The spleen contained, the report states, two well healed, intact parathyroid glands. The second dog had tetany of short duration. It recovered, however, entirely, still lives, and consequently has, the author believes, parathyroids in the spleen which are functionally sufficient.

With the exception of the two cases of Biedl, I find no report of the successful isotransplantation of the parathyroid glands, and besides my own the only successful autotransplantations of these glandules in dogs are, perhaps, the two in puppies reported by Pfeiffer, Hermann, and Mayer. As to the success of the isotransplantations of Professor Biedl, I confess to a little surprise in view of the fact that it is quite out of accord with what one, *a priori*, would expect, and is also at variance with the results of my experiments; and for the following reasons. (1) Parathyroid deficiency was not created by Prof. Biedl either prior to or at the time of the transplantations—not until "after a time." (2) *Two* of the glandules were transplanted in each instance and *both* are said to have lived and performed their function. If this were the case the dogs must each have been possessed for a time of six functioning parathyroid bodies. (3) The transplanted glandules were "foreign." I can not, with my present views, quite credit the observation that an animal in full possession of an organ or set of organs whose function it is to furnish an internal secretion is capable of sustaining in a functioning state a second organ or set of organs of the same kind, and particularly not when these transplanted organs are of "foreign" origin. (4) The functional proof is lacking inasmuch as the glandules were not excised during life.

The results of the experiments undertaken in the winter of 1906–7, and of a portion of those made in 1907–8 have, previously, been reported in briefest abstract.[1] Since the publication of these reports there have been developments, the result of further experimentation, which should be recorded. The cases of especial interest will be considered in some detail, the remainder recounted in the briefest manner.

[1] *Proceedings of the Path. Soc. of Philadelphia*, 1908, xi, 109; *Proceedings of the Soc. for Exper. Biol. and Med.*, 1908, v, 74.

Sixty dogs, approximately, have been used in this investigation and more than 130 parathyroid glands transplanted. The technique has been precisely that of the operating rooms of the Johns Hopkins Hospital.

Of the first series, performed in the winter of 1906–7, there follow brief notes, with comments, of a selected few of these experiments which, interpreted in the light of my work of the following winter, contribute something to our knowledge.

Dog A. Jan. 15, 1907. *Operation I.* Autotransplantation into thyroid lobes. Excised the four parathyroid glandules, leaving, perhaps, a film almost too fine for ocular demonstration, of the left internal one, on the thyroid gland. Three of the glandules were replaced, in new situations, in the thyroid lobes.

Feb. 25, '07. *Operation II.* Excision of such parts of the thyroid gland as were believed to contain the transplanted bodies. Only one of the autoplants or autografts, was found. It was smaller, harder and less red than the normal parathyroid gland. *Microscopic examination.* The nuclear staining is excellent. The transplanted epithelial body seems altogether normal except that a little reactive fibrous tissue surrounds it and, here and there, in delicate strands, enters it. It is obviously difficult to furnish convincing evidence of functional proof.

As the dog had no symptoms of tetania parathyreopriva after the first operation, we are inclined to believe that the film of parathyroid tissue left on the thyroid gland in the process of shaving off the left internal parathyroid was sufficient to hold them in check until one of the much needed autografts had obtained its new blood supply and resumed its function. That only one of the transplanted glandules lived surprised me at the time of the observation, when I had hoped to find all three of them surviving, but this is the finding which, with my added experience, I should now be prepared for. I am anticipating a little in making at this point the statement that only one of the transplanted parathyroid bodies in a given animal, has in our researches survived; and furthermore that only so much of the successfully grafted epithelial body has lived as seemed essential to prevent pronounced symptoms of tetany, and not enough of it to entirely obscure evidence of hypoparathyroidism, such as a slight fibrillary tongue tremor.

Dog E. Jan. 23, 1907. *Operation.* Autotransplantation into the spleen. The two glandulæ parathyroideæ of the right side of the neck and the superior glandula parathyroidea of the left were excised and planted into the spleen.[8]

[8] The technique of the splenic transplantations is described and illustrated in the first article published by me on the subject of transplantation of the parathyroid glandules in dogs, *Amer. Jour. of Med. Sciences, loc. cit.*

The left thyroid lobe was also excised to insure destruction of the fourth glandula parathyroidea which was not seen.

Jan. 25, '07. Tetany.

Jan. 26, '07. Dead. *Autopsy* negative. Death believed to be due to tetania parathyreopriva.

Dog G. Jan. 28, 1907. *Operation.* Autotransplantation into the thyroid lobes. The internal left parathyroid glandule was excised and reimplanted into the left lobe of the thyroid gland. The superior (external) right parathyroid glandule was implanted into the right thyroid lobe near its anterior border, about 1 cm. above its inferior pole.

Feb. 8, '07. Dog has not been at all well since the operation. Believed to be suffering from parathyroid privation due to the considerable insult to both thyroid glands as well as to the excision of two transplanted bodies.

May 8, '07. One hundred and nine days since the transplantations; killed. *Autopsy.* There is no trace of the transplanted bodies, except a little pigment at the site of the transplantation of each.

This case is cited as evidence favoring the view that the thyroid privation was either insufficient or not sufficiently long continued to insure success for the transplantation. The restoration of function in the maltreated, but non-transplanted glandules, may have taken place too rapidly to admit of the return to life of either of the two transplanted bodies.

Dog H. Feb. 5, 1907. *Operation.* Searched neck carefully and did not find a single parathyroid glandule. It is the only time that I have had this experience. Probably to be explained, in part at least, by careless operative technique.

Feb. 8, '07. Has not been well since the operation. Is very thin. Slight fibrillary tremors.

The experiment is cited to show what I observed very frequently in my experiments in transplantation and extirpation of the thyroid gland in 1888–9,[9] that mere handling of the thyroid lobes may give rise to symptoms of parathyroid privation. Manipulation, not excessive, of the thyroid gland, in dogs, has several times in my experience produced fatal tetany.

Dog N. Apr. 10, 1907. *Operation I.* Isotransplantations. Into the spleen were placed five parathyroid bodies taken from three or possibly four dogs. The transplantations all took place very smoothly by means of the needle and thread method described in a previous article (*l. c.*). Some of the glandules were kept in blood serum for, possibly, half an hour. The last obtained were, however, promptly transplanted. Parathyroid deficiency was not created.

[9] An Experimental Study of the Thyroid Gland of Dogs, with Especial Consideration of Hypertrophy of this Gland, *Johns Hopkins Hospital Reports*, 1896, i, 373.

Apr. 16, '07. *Operation II.* Panthyroidectomy performed on both sides in such manner as to remove or destroy the blood supply of the parathyroids. Dog was rather weak before this operation. His cage companion of a few days before had died of distemper. I was not aware of this until after this operation.

Apr. 17, '07. Dog has tetany; evidenced by general tremors and rigidity.

Apr. 18, '07. Barely able to stand; very emaciated. The tremors observed yesterday are not demonstrable today. Trousseau's phenomenon cannot be elicited. Has cough, and from the nose is a discharge of blood-tinged mucus. Vomits. Gave hypodermic injection of one phial (ℳ xii) of a protonucleid extract of parathyroid gland kindly sent me by Dr. Beebe for the patient so often referred to by me. Dog is being fed chiefly on milk.

Apr. 19, '07. No tremor nor spasms nor stiffness and dog runs about with surprising activity considering the great emaciation. Cough persists; also the discharge from nose.

Apr. 20, '07. 1.30 P. M. Dog much worse. The signs of tetany have all returned. Fell in tonic spasms with all four legs drawn to belly and toward midline, reminding one of the comparison to the obstetrician's hand in cases of tetany in the human subject. Second injection of nucleoproteid (Beebe).

Apr. 22, '07. Dog weaker than ever and greatly emaciated. Can, however, stand and totter about. Laps water in characteristic way, for minutes at a time, without lowering the level of the water in the basin.

Apr. 23, '07. Died last night. *Autopsy.* Each pleural cavity contained about one pint of blood-stained, slightly purulent fluid. Peritoneal cavity negative. The spleen was carefully sliced. One little necrotic body, smaller than and with the color of a mustard seed was found in the spleen near its surface, otherwise only a blood stain here and there remained as traces of the transplantations made two weeks ago.

The protonucleid extract (Beebe) was used in two of the dogs with tetany and with some effect, it was thought. The above dog lived six days after removal of his parathyroid glands, a long time considering the finding in the pleural cavities. Possibly the absorption of the five isoplants as well as the administration of the Beebe extract influenced the result. It can well be objected in this case that the glandules, or at least three of them, used in the isografting were not transferred to the spleen with sufficient promptness, and that their failure to survive was due in part to distemper and the thoracic complication.

In operations involving a large number of dogs and so many operating tables I was assisted by my house surgeon, Dr. Sowers, and by several other members of my hospital staff who made possible such an undertaking without delay and with the observance of a technique almost above criticism. Quite a large piece of the

thyroid gland was usually excised with its internal epithelial body to support and thereby facilitate the cutting of the latter; but only a small piece of the former remained in the isograft as prepared for the transplantation. The transplantations were on the whole very smoothly done.

Dog O. Apr. 24, 1907. Heavy, vigorous, young fox terrier. Estimated age one and a half years. *Operation I.* Isotransplantations, into spleen, of eight parathyroid glandules obtained from five dogs. The glands to be grafted were given a raw surface when external, cut with thyroid tissue when internal and each kept in the blood serum appertaining to it. Parathyroid deficiency not created until nineteen days later.

Apr. 25, '07. Dog is doing well.

Apr. 26, '07. Has appetite and runs happily about.

May 7, '07. Perfectly well and vigorous.

May 13, '07. *Operation II.* All thyroids with parathyroids excised.

May 14, '07. 8 A. M. Already severe tetany. 2 P. M. Severe symptoms have abated. Has tongue tremors still and laps water in the characteristic way. Refuses food.

Apr. 15, '07. 4.30 P. M. Extremities quite rigid. Fine tremors. Beebe's nucleo-proteid injected (about 10 minims). 4.45 P. M. Dog seems definitely brighter. Laps a little milk effectually. Stands up quite naturally. Fine tremors of tongue and of shoulder muscles persist. Refuses meat.

Apr. 16, '07. Found dead. *Autopsy* negative. Peritoneal and thoracic cavities normal. Spleen very carefully examined. The isografts have all been absorbed. Not a trace of them remains. At the surface of the spleen within the area of transplantation, a tiny white speck which we thought might possibly be the remains of a graft was preserved for microscopic examination. It proved to be nothing but fibrous tissue.

This case is one which, perhaps, above all others convinced me of the inadvisability at this time of further attempts at transplantation without the production of a parathyroid deficiency. I had not as yet ascertained that for isografts the likelihood of survival is, to say the least, very little. I have hoped for an opportunity to test isografting between dogs of one litter.

Results (winter of 1906–1907);[10] *Autotransplantation.*—A de-

[10] In 1906–07, I was assisted by members of my house staff and by medical students, especially Messrs. Chesnutt and Dinsmore; this year Dr. Hennington has rendered me great service in the operative and kennel work. The vicissitudes have been many—pneumonia, distemper, accidents, and escapes. On one occasion the laboratory was broken into, presumably by a discharged employee, and many of my most prized dogs set at large. Some of these dogs were awaiting the ultimate functional test of the transplantation after two or more operations.

gree of parathyroid deficiency is of necessity created in the autotransplantations. Of five autografts into the thyroid lobes of three dogs, two were successful (macroscopic and microscopic proof). Of eight autografts into the spleens of three dogs, one only succeeded (macroscopic proof). In no instance was functional proof of the success of these transplantations obtained. Such proof can not, of course, be convincingly obtained in the cases of transplantation into the thyroid because of the lack of certainty that no parathyroid tissue except that transplanted remains at the time of the final operation, at which well nourished thyroid tissue, sufficient to insure the life of the transplanted parathyroid gland, must be left.

Isotransplantation.—In five cases Dogs (K, L, M, N and O), two, seven, five, five, and eight parathyroids, respectively, were transplanted into the spleen. In only one dog (K) was a parathyroid deficiency created. In no instance was the transplantation successful; furthermore, tetany supervened and death occurred just as promptly, after removal of the thyroids and parathyroids in the neck, in these dogs with so many intrasplenic isoplants, as in the ungrafted dog. Hence we conclude that, in this instance, life was probably little, if at all, prolonged by the absorption of the parathyroids transplanted into the spleen.

The Technique of the Transplantation behind the Rectus Abdominis Muscle.—In the earlier transplantations the autografts, whether in the mid-line or behind the Musculus rectus abdominis, were, as a rule, placed either at the bottom of the wound or in its immediate vicinity with the view that, in the ultimate search for them, proximity to the wound would facilitate their discovery. It was, however, soon noted that the glandules behind the rectus muscle could distinctly be seen through the several layers of delicate and trasparent fascia, namely, the posterior sheath of the muscle, the subperitoneal fascia and the peritoneum. They would instantly be detected from a mid-line wound on reflecting outward the abdominal wall. It became then our established procedure (1) to make an incision 1 cm. or less in length through the aponeurosis in front of the Musculus rectus abdominis over the inner edge of this muscle; (2) with the blades of our fine "mosquito-forceps," to liberate this border of the muscle from the mid-line fascia; and (3) with

a second pair of this forceps, to introduce the glandula parathyreoidea between the widely separated blades of the first, to as high a point as feasible behind and within the sheath of the rectus muscle. In this manner the autograft is carried an inch or two above the line of incision through the anterior sheath of the muscle and well out of the sphere of influence of a possible drop or fraction of a drop of blood. The glandules successfully transplanted in this wise produced, except in one instance, no opacity of the fasciæ through which they were to be observed, and, at a glance, were discoverable under the glistening lining of the abdominal wall. The incision through the aponeurosis anterior to the Musculus rectus abdominis was closed with one mattress suture of China bead or the finest black silk. Over this the skin was brought together with sub- and intracutaneous and epithelial stitches. The abdominal wounds healed *per primam* without exception. The wounds of the neck were very carefully closed with continuous layer-sutures of the finest black silk (A or O) in a milliner's needle. Oozing points were included in the sutures, of which there were usually five or six rows—one each for (1) the mid-line muscles; (2) the deep fascia and muscle sheath; (3) the platysma and deep layer of superficial fascia; (4) the deep cutis and superficial layer of superficial fascia; (5) the mid-cutis; (6) the epithelium. The stitch which we designate as "epithelial" is particularly useful in the surgery of animals. It excites no irritation and includes so little more than the cuticle that it is finally shed with the latter, unless it, previously, be peeled or ripped off. The sewing with the fine straight needle may be accomplished with unusual rapidity. Aseptic precautions, thanks to the skillful assistance of Dr. C. W. Hennington, were throughout as perfect as we could make them. With perhaps five exceptions the neck wounds healed throughout *per primam.* It was chiefly in connection with the second operations carried out in the line of the original wound that the suppurations occurred.

Dog No. 1. Feb. 3, 1908. *Operation I.* Excised the superior, right parathyroid gland and transplanted it into the right rectus abdominis muscle. There was no bleeding. Excised the right thyroid lobe entire. Layer suture with black silk, collodion dressing.

Feb. 9, '08. Convalescence without incident. Both wounds healed *per primam.*

Feb. 10, '08. *Operation II.* Transplanted a small, freely movable parathyroid gland found resting on the thyroid gland, about midway between its poles, into the left rectus abdominis muscle. Excised the left thyroid gland, entire. The dog is now probably deprived of all parathyroid glands except the two transplanted ones.

Feb. 11, '08. Dog fairly well. No fibrillary tremor of tongue. No muscle twitching.

Feb. 12, '08. No definite sign of tetany.

Feb. 13, '08. 10 A. M. Dog very ill. Respirations 160–180 per minute. Pulse too rapid to be counted. Blood-tinged foam at mouth. Walks a few steps then falls and crawls with head close to the floor. Typical tremors. About 2 P. M. great improvement and at 6 P. M. nearly complete recovery. Pulse 120.

Feb. 14, '08. 10 A. M. Quite ill. Fibrillary tongue tremors, and clonico-tonic contractions of temporal and other muscles; characteristic gait. 5 P. M. calcium lactate, 4 grammes, in water administered per stomach tube by Dr. Voegtlin. Marked relief after one and a quarter hours.

Feb. 15, '08. Condition about as it was yesterday, before the exhibition of the drug. Calcium lactate again administered, and with difficulty, for attempts to pass the stomach tube excited convulsions. Improvement.

Feb. 16, '08. Sunday. Dog observed but once. Little if any better.

Feb. 17, '08. Definitely worse. Very ill. Calcium lactate administered for the third time. Prompt and marked improvement.

Feb. 18, '08. Better. Fibrillary tremors of tongue. Calcium lactate, fourth dose. Beneficial effect.

Feb. 19, '08. Improved. Tongue tremors persist, but are fainter. Calcium lactate, fifth dose.

Feb. 20, '08. Dog much better. Calcium lactate, sixth dose. Benefited.

Feb. 21, '08. Fairly well. Trace of tongue tremor.

Feb. 24, '08. No calcium for four days. Dog not so well. Marked tongue tremor and slight general muscular twitching. Calcium withheld.

Feb. 25, '08. Better. Tongue tremors.

Feb. 27, '08. Improving, active. Slight tongue tremors.

Mar. 2, '08. Much improved. Gaining in weight. Tongue tremors persist,

Mar. 6, '08. Fairly well. Tongue tremors very slight.

Mar. 11, '08. Quite well. Tongue tremors still demonstrable. It is twenty days since the last dose of calcium was given, and thirty days that the dog has been deprived of all parathyroids except the ones transplanted.

Mar. 20, '08. Dog is becoming much stouter, seems in excellent health. Frisks about as if life were enjoyed. But faint tongue tremor is, still, always to be seen.

Mar. 30, '08. Dog is strikingly plump. Faint tongue tremors, and rather general fibrillary twitchings are still manifest on careful observation.

Apr. 4, '08. Dog "fat" in the extreme. Contrasted with the other dogs, it seems quite certain that myxœdema has developed.

Apr. 9, '08. Normally active and apparently healthy. Continually increasing in size. Thickening of skin and subcutaneous tissue particularly conspicuous in nape of neck. Trace of tongue tremor usually visible.

Apr. 23, '08. Myxœdema increasing steadily. Dog somewhat deformed by it.

June 11, '08. Dog's plumpness is monstrous and rather uncanny. His hair is falling out. Activity, spirits and general health are good.

June 15, '08. Transferred to Drs. Voegtlin and Strouse for observations and experimentation during the summer.

Nov. 1, '08. This dog died during the past summer.

The cause of death was undiscoverable at autopsy. Dr. Voegtlin, who observed him carefully, is positive that tetania parathyreopriva did not at any time develop. He will publish a photograph which he took of the animal to show the state of excessive myxœdema, which has so rarely been produced in the dog. The preparations containing, presumably, the autograft and the subaortic accessory thyroid glands were, through a misunderstanding, destroyed by a servant to whom they had been, for a moment, intrusted. We are, therefore, in doubt to which of the two autoplants, in case only one survived, the prevention of tetany is to be ascribed.

It is conceivable, of course, that portions of two or more autoplants may live. This should, I think, be more likely to occur when two glandulæ parathyroidiæ are transplanted at the same time I am inclined to believe that a very considerable parathyroid privation, greater, perhaps, than is ordinarily produced by the excision of the two parathyroids of one side may be essential to the success of the transplantation.

Dog No. 2. Feb. 3, 1908. *Operation I.* Total right thyroparathyroidlobectomy (right panthyroidectomy[11]) and transplantation of the right internal parathyroid gland in the mid-line, just external to the peritoneum.

Mar. 10, '08. Dog has been perfectly well since the operation. Wounds healed *per primam*. *Operation II.* Total thyroparathyroidectomy on left side and transplantation of the left internal parathyroid gland in the midline, præperitoneally. Dog has now, presumably, no parathyroid glands except those transplanted.

Mar. 15, '08. Has been perfectly well since the operation. Has manifested no signs of tetania parathyreopriva. Observed once or twice daily by Dr. Hennington and repeatedly by me.

Mar. 18, '08. A. M. Observed by Dr. Hennington, who, on careful examination of his tongue, found no trace of tremor.

Mar. 19, '08. Found dead in cage late yesterday afternoon. 12 M. *Autopsy.* Blood in nostrils, abdomen slightly distended, normal peritoneal cavity. Both lungs quite solid, and on section, dark red-black and infiltrated with blood-stained exudate. In the search for the transplanted parathyroid glands two

[11] A word proposed for the sake of brevity, but with some misgiving and disapproval

very small brown-red bodies were found in the mid-line subperitoneally, in the line of the cicatrix, and perfectly isolated from all tissues which might resemble them. They had the color which, as we learned later, is common to the successfully transplanted parathyroid glands. They have, of course, been preserved for microscopic examination (Path. No. 8764).

Death was quite certainly caused by pneumonia, and the time *post operativum* is too brief for conclusions. The absence of signs of tetany, however, for eight days after the second operation, and the fact that the transplanted epithelial bodies had not become necrotic is evidence in favor of the success of the transplantation. This is the only instance, in our experiments, in which, apparently, two autografts, at least part, have survived. I regret that sections have not as yet been made.

Dog No. 3. Feb. 5, 1908. *Operation I.* Autotransplantation of the right external parathyroid into the right rectus abdominis muscle. Removal of the right lobe of the thyroid with the internal parathyroid gland. In Dogs Nos. 1 and 2 the rectus muscle was prepared for the transplantation before the parathyroid body was excised, in the belief that the time of exposure of this body to the air might be shortened. But a very few seconds, if indeed any, were saved by this procedure and hence it was abandoned. The epithelial bodies to be transplanted were thenceforth kept in the blood of the wound, sometimes within, at other times without the body, the time required for the transfer rarely exceeding a few seconds. In case of the slightest bleeding a new pocket in the rectus would have been made.

Feb. 20, '08. Dog recovered from Operation I without incident. Both wounds healed *per primam. Operation II.* Procedure, precisely as described above, carried out on the left side, except that the internal parathyroid was used for transplantation instead of the external glandule. The dog is thus deprived of both thyroid lobes and for parathyroids is reduced to the two transplanted glandules.

Feb. 23, '08. Dog seemed fairly well for forty-eight hours *post operativum.* No signs of tetany could be detected. Found dead to-day. *Autopsy.* No blood in nostrils. Trachea and bronchi contain blood-stained frothy mucus. Right lung airless, heavy, congested. On section there exudes a bloody exudate. Sections made of the lung indicate a pneumonic process. The autopsy findings are otherwise negative. The parathyroid transplanted Feb. 5 was found on the posterior surface of the right rectus muscle much reduced in size and of a yellowish-brown color. The second gland, transplanted only three days before death, was surrounded by blood clots which may have been responsible for its death as well as, in part, for the death of the animal.

Dog No. 4. Feb. 5, 1908. *Operation I.* Right superior parathyroid gland transplanted præperitoneally in the mid-line, above the umbilicus. Removal of right lobe of thyroid gland with its internal epithelial body.

Feb. 29, '08. Dog perfectly well since operation. *Operation II.* Excised left

thyroid lobe with its parathyroid glandules and transplanted, præperitoneally, in the middle line, the left superior and inferior parathyroids, the latter being in two parts, contiguous, and each about 1.5 mm. in diameter. Dog deprived of both thyroid lobes and all parathyroids except the transplanted three.

Feb. 22, '08. Dog disinclined to come out of cage. Wags tail and stands up when petted. Faint trace of fibrillary tongue tremor.

Feb. 23, '08. About as yesterday—a little worse, perhaps.

Feb. 24, '08. Cough; muco-purulent discharge from nose which dog constantly licks. Licking of the nose, even when dry, is a characteristic symptom in tetania parathyreopriva. Faint fibrillary tremor linguæ. Other muscles not demonstrably affected. Dog decidedly worse, but we have the impression that his illness is not altogether due to hypoparathyroidism.

Feb. 25, '08. Calcium lactate, one dose, was given without definite effect.

Feb. 26, '08. 10 A. M. No tremor, but dog much weaker and very thin. Muco-purulent discharge from nose continues. Died about 3 P. M. *Autopsy.* Abdominal cavity negative. Wounds healed *per primam.* Barely a trace remains of the transplanted parathyroid glands. Left lung negative, except for small central induration. Right lung, lower lobe, consolidated and in state of hepatization beginning to resolve. Pneumonia must have hastened the death of this dog, and possibly was in a measure responsible for the failure of the transplantation.

Dog No. 5. Feb. 5, 1908. *Operation I.* Autotransplantation. Right superior parathyroid glandule transplanted behind right rectus muscle. Excised right thyroid lobe with its internal epithelial body.

Feb. 20, '08. Dog has been well since Operation I. Both wounds healed *per primam. Operation II.* Left superior parathyroid not found. Left internal glandule planted behind left rectus muscle. Left thyroid lobectomy. Dog reduced to the transplanted glandules.

Feb. 22, '08. Dog seems fairly well.

Feb. 23, '08. Not so well. Panting respiration.

Feb. 24, '08. A. M. Explosive expiratory efforts frequent. Tremors not demonstrable. Neck wound broken down. This is the first instance in these experiments of failure to heal *per primam.* P. M. Tongue tremors very pronounced.

Feb. 25, '08. Dog died about 2 P. M. Calcium lactate not administered in this case. *Autopsy.* Thoracic cavity negative except for a remarkable finding in the anterior mediastinum. There is an infiltration of blood, only three or four drachms in quantity, between the pleural leaves and lying chiefly on the pericardium, entirely outside of this sac. The blood-infiltration extends into the left neck to a point hardly above the subclavio-jugular junction. There is nothing to indicate that the blood gravitated from the neck to the mediastinum, for the former is perfectly healed and unstained, and the mediastinal blood staining fails to reach the neck wound by 3 or 4 cm. The mediastinal blood is not infected. It has excited no reaction, and the contiguous serous membranes have their natural lustre. I conclude that some traumatism, a fall or a self-inflicted blow on the mediastinum during recovery from the anæsthetic was responsible for this lesion. The blood extravasation was too slight, it seemed to me, to have been a factor in the death of this animal. A very careful search

for the transplanted glandules by Dr. Hennington and myself failed to reveal a trace of either except a minute area of tissue staining. *This is the first dog of the series of autotransplantations, behind or in the musculus rectus abdominis, to die of uncomplicated tetany.*

Dog No. 6. Pregnant bitch. Feb. 6, 1908. *Operation.* Autotransplantation. The right superior parathyroid gland planted into the right rectus abdominis muscle. Excision of right thyroid lobe.

Apr. 5, '08. Well since operation. Wound suppurated. Gave birth to-day to five pups. No evidences of hypoparathyroidism during pregnancy or since delivery.

Apr. 7, '08. One of the pups, female, found dead. Each thyroid lobe measures 1 cm. in its vertical and 4 cm. in its transverse diameter. The lobes are connected by an isthmus covering one ring and two interannular spaces. The left superior (external) parathyroid is distinctly seen, though very minute. Weight of puppy 232 grams. Length from tip of nose to tips of hind feet, 26 cm.

Apr. 9, '08. During the past two days the four remaining pups have died. They had increased little if any in size. The average weight of the thyroid gland in each of these was .055 grams; the average length of the lobes 8 mm. and average width 3.5 mm.

Apr. 11, '08. Dog under observation. Retained for completion of the experiment.

Dog No. 7. Feb. 6, '08. *Operation I.* Autotransplantation. The left external parathyroid, situated as low as the middle of the thyroid lobe at its intero-posterior border, was transplanted behind the right rectus abdominis muscle just above the umbilicus. The right parathyroids were not found. Both thyroid lobes were considerably damaged in the search for the abnormally situated parathyroids; neither lobe was excised. The circulation of the unexcised parathyroids was probably much interfered with and thus a *considerable parathyroid privation caused, a factor in my opinion essential to the success of the transplantation.*

Feb. 7, '08. 9 A. M. Dog feels badly, whereas the three other dogs operated upon for autotransplantation about the same time yesterday seem perfectly well. He not only refuses to leave cage, but lies down immediately when placed on the floor of the room. His gait on walking and trotting is uncertain. No fibrillary tremors of tongue. Pulse 152. 4 P. M.. Pulse 128.

Feb. 10, '08. Has been slowly improving since last note.

Feb. 15, '08. Seems perfectly well. Have at no time observed positive symptoms of tetania parathyreopriva, but believe that he suffered from hypoparathyroidism for several days after the operation.

Feb. 20, '08. *Operation II.* Both thyroid lobes excised and, presumably, the circulation of any remaining parathyroid destroyed. The wound was left open on account of a fine fistulous track in the cicatrix from the first operation.

Feb. 22, '08. Dog has been quite well since operation. No signs whatever of hypoparathyroidism.

Feb. 26, '08. Dog in fair health and spirits. On most careful and prolonged examination very faint fibrillary tongue tremors were observed. Hence the autoplant must have "taken," and, presumbly, the parathyroid glandules excised in the double thyroidlobectomy were defunctioned by the first operation.

Mar. 2, '08. Dog is lively, quite well and hungry. No signs of tetany although I have the feeling that he is suffering from very slight parathyroid privation.

Mar. 3, '08. Quite well. *Operation III.* Laparotomy, to explore the posterior surface of the right rectus muscle *and if possible obtain functional proof of the success of the transplantation.* The transplanted glandule was at once recognized and excised. It was apparently full sized, shimmering clearly through the peritoneum. Its relation to the surrounding tissues, so far as reaction in them is concerned seemed to be precisely that of the external parathyroids in the neck. It could be slid about as freely as when in its normal position. I was unable to detect any departure from the normal color of the glandule. In this respect it differed from the transplanted glandules, observed before and more frequently later, in which, although functioning, partial absorption and invasion by fibrous tissue had taken place. In these the red hue was toned by yellow and brown.

Mar. 4, '08. Dog ill, but no tremors observed.

Mar. 5, '08. Very ill. Complete picture of tetania parathyreopriva. *Operation IV.* Isotransplantation of three parathyroid glands behind the left rectus abdominis muscle. Lactate of calcium exhibited.

Mar. 6, '08. Dog found dead this A. M. *Autopsy.* Negative as concerns complications. Death attributable to tetania parathyreopriva. A section of this unusually normal-looking autograft is illustrated in Plate I. That the drawing in Plate I seems inferior to that in Plate II is due to the fact that the transplanted parathyroid from this dog (No. 7) was overheated in xylol.

Dog No. 8. Feb. 6, 1908. *Operation I.* Autotransplantation. The right superior glandula parathyreoidea consisted of two distinct portions quite equal in size; the one, the gland proper, the other, a cyst closely attached to and part of it. The right thyroid lobe was not excised, but it became necessary in the course of its investigation to ligate the vessels at its inferior pole. From the left thyroid lobe, also not removed at this operation, an unusually large internal parathyroid gland was excised and transplanted præperitoneally in the middle line.

Feb. 20, '08. Uneventful convalescence. No suggestion of tetany. *Operation II.* Both thyroid lobes excised in such manner as to include the remaining glandulæ parathyreoideæ. The dog is now reduced to one autoplant.

Feb. 22, '08. Well.

Feb. 23, '08. Well.

Feb. 24, '08. Not quite so well, but signs of tetany not discoverable. Wound healed *per priman.*

Feb. 26, '08. Dog becoming weak and thin. Muco-purulent discharge from nose. Trace of tongue tremor.

Feb. 27, '08. Condition little changed. Tongue tremor questionable. Discharge from nose a little less profuse, seemingly.

Feb. 28, '08. Brighter. Eats with some avidity. Barely perceptible tongue tremor.

Mar. 2, '08. Much better, gaining in weight. Eyes clear, nasal discharge has ceased. Faint fibrillary tremors of tongue and occasionally also of temporal muscles.

Mar. 20, '08. Dog increasing in size. Myxœdema (?). Mucous membranes pale; slight tongue tremors. Appetite good.

Mar. 30, '08. No conspicuous change in condition.

Apr. 4, '08. Tongue tremor not observable to-day. The myxœdema has been increasing.

Apr. 9, '08. Seems quite well. Is awkwardly and unpleasantly plump. No tremors.

Apr. 23, '08. Hair becoming somewhat thinner.

June 11, '08. Myxœdema has increased. Hair is beginning to fall out. State of health apparently unchanged. Transferred to Drs. Voegtlin and Strouse for the summer, who will later report the results of their observations, and of their experiments with three of the myxœdematous dogs of this series.

Dog No. 9. Feb. 6, 1908. *Operation I.* Autotransplantation. Right internal glandula parathyreoidea planted behind musculus rectus abdominis. The superior glandula parathyroidea is very small indeed and in two parts. The right thyroid lobe was excised.

Mar. 24, '08. Autotransplantation behind left rectus of a low-lying superior glandula parathyroidea. A second left glandula parathyroidea was very near the one removed, on the outer surface of the thyroid lobe, below its middle. Left parathyroidectomy. Dog is reduced to the two transplanted parathyroid glands.

Mar. 25, '08. Dog seems unaffected by the operation. Is as lively as ever.

Mar. 26, '08. About as usual. Faint tongue tremors.

Mar. 27, '08. Tongue tremor seen only at times and with difficulty.

Mar. 30, '08. Slight tongue and also temporal tremors. Dog not so well.

Apr. 1, '08. Better. No signs of parathyroid privation.

Apr. 4, '08. In unusually good spirits. No tremors.

May 9, '08. Seems very well. No tremors. Has gained in size.

May 23, '08. Abnormally stout. Myxœdema (?).

June 11, '08. Increasing in size. No tremors. Health seems good. Transferred to Drs. Voegtlin and Strouse for the summer.

Dog No. 10. An old dog. Feb. 10, 1908. *Operation I.* Autotransplantation. Right superior glandula parathyroidea transplanted into præperitoneal pocket, just above umbilicus. Excision of right thyroid lobe (right parathyroidectomy). The transplanted body is so large that I questioned for a time its nature but finally decided that it must be a parathyroid gland. Its edge was characteristically sharp, and, a little softer than usual, it resembled in consistence and form the human glandule.

Feb. 27, '08. *Operation II.* The left internal glandule was transplanted præperitoneally in the middle line below the navel. The left superior glandula parathyroidea is very small. Left panthyroidectomy. Dog is now reduced to the two transplanted glandules.

Feb. 28, '08. Faint tongue and temporal tremors.

Feb. 29, '08. No tremors observed.

Mar. 1, '08. No tremors.

Mar. 2, '08. No tremors. Wounds healed *per primam.* Dog better.

Mar. 5, '08. A. M. Dog jumps about quite happily in his cage and seems

fairly well. P. M. Dog is very ill. Has general muscular twitching and presents the picture of tetania parathyreopriva.

Mar. 6, '08. Dog died during night. *Autopsy.* Made and dictated by Dr. Hennington. Heart and lungs normal. On opening the peritoneal cavity an extravasation of blood into the omentum was observed and, on more complete exposure, a large round worm (*Eustrongylos gigas* or *Dioctophyme renale* (?)), 90 cm. long and 1 cm. in diameter, presented itself free in the peritoneal cavity in the neighborhood of the spleen. It was still alive and on being placed in warm water, executed slight movements. The intestinal peritoneum was thickened and granular-looking. The parietal peritoneum presented in places small, indefinitely circumscribed, roughened areas. More careful examination of the omentum showed that the extravasated blood followed the ramifications of the blood vessels. The liver presented on its surface whitish nodules one to three millimeters in diameter. On section there seemed to be a great increase of fibrous tissue in this organ. The surface of the spleen was slightly roughened; pancreas apparently normal. Stomach dilated with gas. The intestines, large and small, showed no abnormality. Mesenteric glands enlarged. Kidneys quite normal in appearance. The transplanted parathyroids were much changed in appearance and probably necrotic.

The remarkably sudden and very late appearance of the tetany in the above described dog is worthy of comment. This dog was aged. The dogs of this series were, with the exception of this one, quite young—estimated between one and two years of age.

Dog No. 11. Feb. 10, 1908. *Operation I.* Autotransplantation. The right superior glandula parathyreoidea, rather small, was transplanted into the right rectus abdominis opposite the umbilicus.

Feb. 27, '08. Dog has been well since Operation I. *Operation II.* The left superior glandula parathyroidea imbedded about 3 mm. below the upper pole of the thyroid, was transplanted through the fibers of the left rectus abdominis muscle on the posterior sheath of this muscle. Excision of entire left thyroid lobe. The dog is, presumably, reduced to the two transplanted parathyroid glands.

Feb. 28, '08. No tremors.

Feb. 29, '08. No tremors.

Mar. 2, '08. No tremors. Dog seems fairly well.

Mar. 30, '08. Dog has been, apparently, perfectly well since last note.

Apr. 23, '08. Well. Has gained considerably in weight.

June 11, '08. *Operation III.* The autoplants were very thoroughly searched for and only the left one found. This little body did not shimmer through the peritoneum as the transplanted bodies usually do but, projecting slightly, could be felt as a hard round nodule. On section it presented a kind of hilus of connective tissue, and at the cortex five minute brownish dots, separated from each other by a little fibrous tissue, were seen. These dots had the color which seems to characterize the transplanted thyroid and parathyroid glands which have been

partially absorbed and invaded by connective tissue. I have already described this color as a little less red than the normal and as having a browner tinge with a suggestion of yellow.

The dog died of tetania parathyreopriva on the second (or third?) day after the third operation, the operation of functional test.

June 16, '08. The autoplant has been very carefully cut and all the sections preserved. They cover fifteen slides. On each slide are ten sections. Parathyroid tissue appears in none of the sections except those mounted on slides, Nos. 14 and 15, and in all of these (vid. Plate II).

This case is of particular interest proving, as it does, that a very minute piece of parathyroid gland may be sufficient to prevent all objective symptoms of hypoparathyroidism.

Of the macroscopically visible brownish-red dots of the successfully transplanted autograft, the largest proved on microscopic examination to be a fragment of thyroid gland; the smaller dots, barely to be seen with the naked eye, were portions of parathyroid gland, discrete, and separated from each other by strands of connective tissue. The largest of the parathyroid dots is the one depicted so beautifully by August Horn in Plate II. The microscope revealed one or two minute areas of parathyroid tissue, too small to have been detected macroscopically. I regret that there is not time for the making of low-power drawings from these sections.

ISOTRANSPLANTATIONS.

Dog No. 12. March 3, 1908. *Operation I.* Transplantation of the right external and internal parathyroid glands from Dog No. 13 behind the right musculus rectus abdominis, just above the umbilicus. The right superior glandula parathyroidea from this dog was transplanted behind the right rectus of Dog No. 13. Right parathyroidectomy.

Mar. 6, '08. Dog has been well since operation. Is naturally vicious and inclined to bite, hence difficult to examine. No tongue tremors.

Mar. 10, '08. Has been well since last note. 2.30 P. M. *Operation II.* Completed the isotransplantation with Dog 13.

Mar. 11, '08. Dog fairly well but so vicious that a careful examination was not made.

Mar. 12, '08. 9 A. M. Dog is very ill. General twitchings. Calcium lactate administered per stomach tube by Dr. Voegtlin. 4 P. M. Dog wonderfully better. There are no demonstrable twitchings.

Mar. 16, '08. Has been treated daily by Dr. Voegtlin with calcium lactate. Dog still suffers from tetany, partially controlled, however, by the drug.

Mar. 19, '08. Slight fibrillary tremors of the tongue still present but animal looks badly and refuses food.

Mar. 21, '08. Little change in dog's condition. Still refuses food and has faint tongue tremors. No general tremors observed. Calcium lactate discontinued.

Mar. 23, '08. Dog is dead. *Autopsy.* Entirely negative. A few drops of pus were found in the neck wound in mid-line under the skin. There was no trace of the parathyroid gland first transplanted. The second isoplant was necrotic.

Dog No 13. Mar. 3, '08. Isotransplantation with Dog 12. Died of pneumonia two days after the final transplantation.

Dogs Nos. 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 and 28 were employed for isotransplantation experiments and in no instance with success. The dogs were taken in twos and the transplantations made as described for the pair Nos. 12 and 13. In no instance did privation symptoms appear after one-sided panthyroidectomy, but they always supervened on removal from the neck of the final parathyroid gland unless pneumonia carried off the dogs before the tetany had time to develop. The transplanted bodies were absorbed (usually leaving a trace of pigment at the site of the transplantation), or partially absorbed or simply necrotic in accord with the time elapsed after the transplantation. Calcium lactate, which was of such great assistance in several of the cases of autotransplantation, did not suffice to prolong, for more than a few days, the lives of the dogs wholly dependent upon the isografts. To Dog No. 12, developing tetany on the second day after the final isotransplantation, Dr. Voegtlin gave the calcium lactate for nine days, and with great improvement at first; but the animal died of tetany on the tenth day after its incidence.

Results (Winter of 1907–1908); Autotransplantation in or behind the Musculus Rectus Abdominis.—Of eighteen autotransplantations in 12 dogs, 7 parathyroids were absorbed or necrotic (Dogs Nos. 3, 4, 5 and 10); 5 to 7 lived and performed their function (Dogs Nos. 1, (2?), 7, 8, 9 and 11)—in Dog No. 2 for a very short time. In only one instance have we observed the survival of two grafts in one animal whether the transplantation was made in the thyroid, the spleen, or behind the rectus muscle. In the exceptional case the dog died on the ninth day of distemper, without symptoms of tetany. The autografts were very much smaller than when transplanted (vid. history of Dog No. 2).

In Dogs Nos. 7 and 11 the functional test was made. On removal of the sustaining autograft both died promptly of tetania parathyreopriva. In Dog 7, on the development of tetany, isografting was tried without success. Three isografts were placed. Dogs Nos. 8 and 9 still await the functional test. The former is sustained by the one autograft, transplanted February 6, 1908, nine months ago. The latter has been reduced to one or other or both of his autografts since March 24, 1908, a period of seven months. Dog No. 8 has been myxœdematous for about eight months. The myxœdema seems to be hardly as pronounced now (November, '08) as it was in June. Dog No. 9 is only slightly myxœdematous, at least as compared with Dog No. 8. Both have an eczema, dependent, probably, upon the thyroid privation. The falling out of hair has not been great. Neither dog on his return to the laboratory yesterday, November 3, 1908, manifested any of the signs of tetania parathyreopriva.

Isotransplantation in or behind the Musculus Rectus Abdominis.—Of 35 isotransplantations in 17 dogs with created deficiency (Dogs Nos. 7, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 and 28), thirty-two parathyroids were either absorbed or necrotic. Three were transplanted only twenty-four hours before the death of Dog (No. 7). In this case isotransplantation (two grafts) was unsuccessfully resorted to twenty-four hours after the supervention of tetany. Deprived of all parathyroids except the one transplanted (an autograft), his dog lived in good health and spirits for twenty-five days, or until, at a final operation, the sustaining parathyroid was removed. There was in this dog, at times, a suggestion of hypoparathyroidism in a barely perceptible fibrillary tremor of the tongue and of the temporal muscles. On removal of the perfectly normal autograft (vid. Plate I) behind the rectus muscle, tetany developed within twenty-four hours. The following day the isografting was made, and without result, as stated (vid. history of Dog No. 7).

We have not as yet determined that one tissue or site is better than another for the transplantation, and do not even know that parathyroid glandules deprived of their blood supply in the course of operation or by experimentation might not, in some cases at

least, survive if left undisturbed in the original situation in the neck. Inasmuch, however, as drainage is usually employed in thyroid lobectomy, and the drain usually carried to the precise situation of the parathyroids, to the deepest part of the wound, the better site for the transplantation of the glandules would be the opposite lobe of the thyroid gland.

Dr. W. L. Moss has very kindly tested the sera of a number of the dogs used in the experiments of isotransplantation. Reactions such as hemolysis and agglutination have not been obtained.

General Consideration.

Isotransplantations without created deficiency were made into the thyroid gland and into the spleen and were uniformly unsuccessful. In the isotransplantations with created deficiency the grafts were, with few exceptions, placed behind the musculus rectus abdominis. In not a single instance in our experience has an isograft lived. An autotransplantation entails necessarily, at least for a time, a deficiency of some degree. When the loss has been of two parathyroids only, as in one-sided panthyroidectomy, the grafts have invariably and promptly been absorbed (Dogs G, 3, 4, 5, 6 and 10). In other words, all the autotransplantations made at the first operation, at which the parathyroid glandules of one side only were excised have failed. In two instances (Dogs 7 and 8) the autograft transplanted at the first operation survived, but in each case a prolonged search for the glandules was made on both sides of the neck at this operation and so great a degree of parathyroid privation brought about that both dogs were ill with symptoms of hypoparathyroidism, presumably in consequence of the damage done to the circulation of the unexcised parathyroid bodies. Dog 7 was used for the functional test, and Dog 8 has been sustained nine months by one parathyroid glandule; he still lives and, except for myxœdema of a very pronounced type, seems in good health. In the two instances in which to the present time operation for the functional test has been made, a living autoplant has been found; in one of these it was the glandule transplanted at the second operation after complete thyroid privation (Dog 11); in the other, although the first,

it was the only graft, and had been transplanted, as I have said (vid. history of Dog 7), after an operation so destructive to the parathyroid bodies as to cause tetany. In another of the successfully grafted dogs (Dog 1), which died during my summer vacation from cause other than tetany (autopsy by Dr. Voegtlin) it was probably the second and not the first graft that lived because the dog had very severe tetany after Operation II and would have died, we thought, if calcium lactate had not been given for several days. Such severe tetany we have seen only after complete parathyroid privation. A mild degree of tetany might conceivably be induced by anæsthetization and operation even though one parathyroid glandule were in a measure performing its function.

Two dogs, with myxœdema, await the functional test (Nos. 8 and 9). In the case of Dog 9, alive and in good health except for thyroid deficiency (slight myxœdema) and sustained by one parathyroid body for the past seven and one-half months, it has not been determined which one of the two autografts survives. That he has slight symptoms of tetany following the second operation does not enable us to arrive at a conclusion, for with only one parathyroid glandule, and that a graft, such slight manifestations of hypoparathyroidism might not only appear after anæsthetization and operation but even persist, as our observations have taught us (Dogs 1, 7 and 8), for months.

In the light of the work of last winter, the re-study of the experiments of the year preceding strengthens the view that a considerable degree of deficiency is essential to the success of the transplantations. For, of the autotransplantations into the thyroid gland and spleen, only those were successful in which as many as three glandules were excised. In the single case of autotransplantation into the thyroid gland in which the deficiency did not exceed one-half of the normal parathyroid supply (Dog G), both autografts, searched for not until three months and twenty days after their transplantation, were completely absorbed.

In only one instance of autotransplantation were all of the parathyroid bodies excised at one operation. In this case (Dog E) the four grafts were placed in the spleen. Death from tetany occurred on the third day after the operation. It is possible that

calcium lactate might have rescued this dog from the list of failures; but the experiment was made in the year preceding this very important discovery of MacCallum and Voegtlin. That this salt was of great service to us in several cases, converting failure into success, there can be little doubt; and hence the query, is not success more likely to attend transplantations in animals such as rats, which may for a long time (weeks or even months), and in a state of chronic hypoparathyroidism, survive total loss of the parathyroid tissue, than in dogs which so commonly succumb before the end of the third day when the parathyroid privation is complete? For the success, then, of the transplantation of a completely extirpated organ the animal must be able to survive the total privation long enough for the grafts to acquire a circulation sufficient for, at least, a partial exercise of their function, unless chemical or other assistance be rendered. If death might be expected to follow complete privation of an organ furnishing an internal secretion, within say twenty-four hours, successful grafting might be impossible unless the period necessary for the "taking," or "catching" of the graft could be bridged over by the administration of doses of the missing gland, or of its chemical equivalent. It is significant that pneumonia occurred only in dogs whose parathyroid glandules had been totally excised.

The experiments have not been, in number, sufficient to establish as tact the necessity for creating a deficiency, but they lend strong support to this view. It is conceivable that the organism may sustain of transplanted parathyroid tissue only enough to prevent symptoms of privation or to preserve life and not an amount sufficient for the maintainance of perfect health. Is it then worth while in the human subject to transplant a parathyroid removed or injured in single thyroid lobectomy, which creates at most only a moderate degree of insufficiency and not enough perhaps to make probable the success of the grafting? What may be true of dogs need not be true of human beings. Nor can we know in a given case that the parathyroids are four in number, of equal size, and regularly distributed. The transplantation into the opposite lobe after thyroid lobectomy would require but a few moments, and should, in my opinion, not be neglected when a parathyroid body has been

cut off from its blood supply. A glandule removed in the course of operation upon the second lobe (an accident which will rarely occur in the future) might, we believe, be more likely to survive transplantation than a glandule excised at the first operation, but only in case parathyroid privation, sustained for sufficient time, had been brought about by the original lobectomy.

Summary.

1. The autotransplantation of parathyroid glandules into the thyroid gland and behind the musculus rectus abdominis has been successful in sixty-one per cent. of the cases in which a deficiency greater than one-half has been created.

2. In no instance has the autotransplantation succeeded without the creation of such deficiency.

3. Isotransplantation has been uniformly unsuccessful.

4. Parathyroid tissue transplanted in excess of what is urgently required by the organism has not lived.

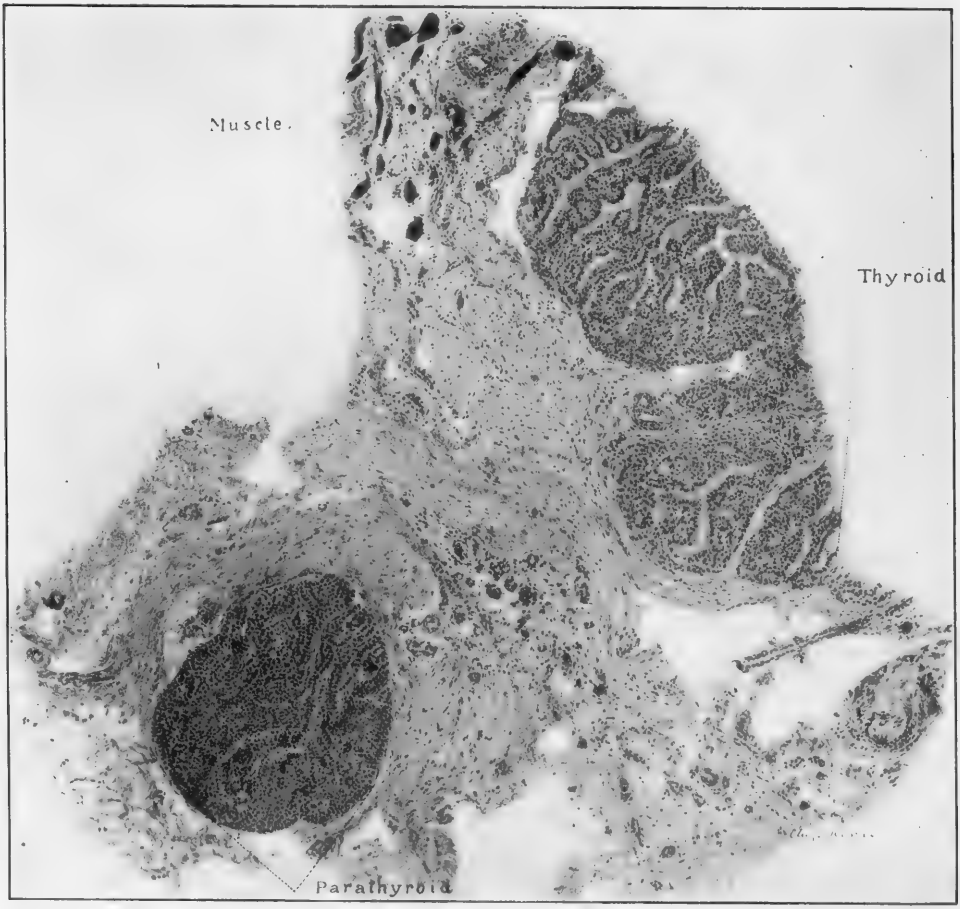
5. One parathyroid autograft may suffice to maintain the animal in good health and spirits for many months and possibly for years.

6. Excised or deprived of their blood supply in the course of operation upon the human subject, parathyroid glands should, in the present state of our knowledge, be grafted, and probably into the thyroid gland.

7. Complete excision of the thyroid lobes in dogs may be well borne for a year or more. The myxœdema which usually has manifested itself within a few weeks has not increased after the first few months. May it subsequently diminish with the hypertrophy of accessory thyroids?

8. Parathyroid tissue is essential to the life of dogs, as has been conclusively proved by the result of excision of the sole, sustaining graft.

There may be found, perhaps, in our experiments, explanation of the fact observed by others (Enderlen, Payr) that, when transplanted, thyroid preserves its integrity less well than parathyroid tissue. In the instances recorded the amount of thyroid gland excised may have been insufficient to make possible the fullest success of the transplantation; and particularly so when we consider the extent of the hypertrophy of which the thyroid gland seems capable.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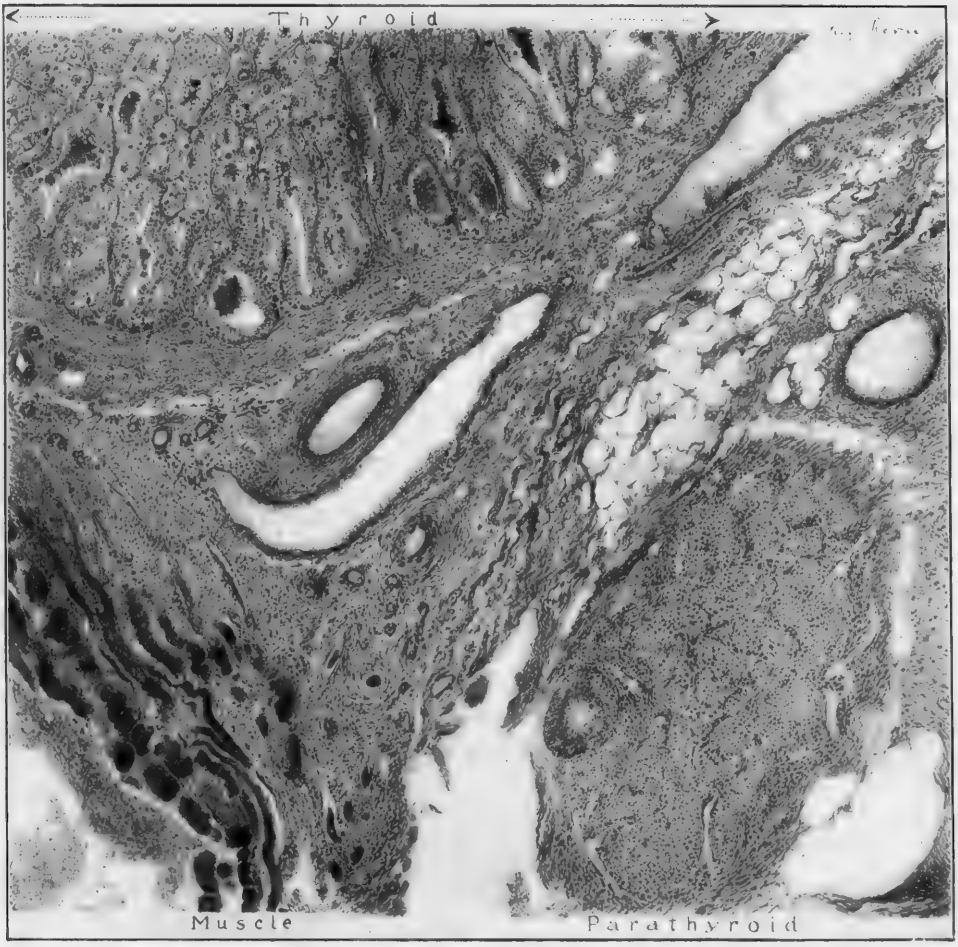
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Explanation of Plates.

Plate II. Enlarged 125/1. The autograft from Dog No. 7, excised at the operation of functional test, one month after its transplantation. Death from tetany promptly followed its excision.

Plate III. Enlarged 90/1. The autograft from Dog No. 11, excised at the operation of functional test, four months after its transplantation. Death from tetania parathyreopriva promptly followed its excision.

THE CATALYTIC ACTIVITY OF THE BLOOD IN RELATION TO (1) THE FUNCTIONAL SUFFICIENCY OF THE KIDNEYS; (2) PERITONITIS.[1]

By M. C. WINTERNITZ.

(*From the Pathological Laboratory of the Johns Hopkins University.*)

In a recent article[2] on the occurrence of catalase in human tissues and its variations in diseases, it was shown that in various conditions individual organs were decidedly decreased in their catalytic activity. For example, a tuberculous lung showed a decrease which varied in direct proportion to the amount of caseous degeneration. This could be easily explained since in such a lung the living tissue is decreased and its blood content is diminished. This latter factor must be emphasized because the stroma of the red blood cells is very rich in catalase. As a consequence we find in such conditions as the red hepatization of pneumonia and in red infarcts, a great increase in the catalytic activity of the diseased tissue. On the other hand, although the caseous lung has a decreased activity, the blood and other tissues show no corresponding change except where there is a secondary anæmia. Here the decrease explains itself, there being fewer red blood cells in the circulation.

In a large series of cases of nephritis, the kidney always showed a decrease in catalytic action. It was noteworthy, however, that the catalytic activity not only of the kidney but of the blood and other organs as well was decreased. This decrease varied to a great extent with the pathological findings, but it varied much more uniformly with the intensity of the clinical picture. Thus in the cases which clinically manifested uræmic symptoms, there was the greatest decrease. In these cases the kidneys always showed extensive degenerative changes which varied between the two extremes of the

[1] Received for publication October 30, 1908.

[2] Winternitz and Meloy, *Jour. of Exper. Med.*, 1908, x, 759.

historic large white and the small granular types, while several showed acute changes superimposed upon chronic ones. The decreased activity of other organs, remote from the kidney was most significantly illustrated where some pathological change was present which under other conditions would have increased the activity of those organs. In several cases, for instance, there was a terminal pneumonia in the stage of red hepatization, and in these the lung showed much less activity than was usually found in red hepitization. Since the catalase of blood is contained in the stroma of the red blood cells, and these are unequally distributed in the post-mortem clot, results obtained with post-mortem blood were considered unreliable. For this reason and also to determine whether the catalase of the blood was reduced during life in nephritis, the blood of several nephritic patients in the wards of the Johns Hopkins Hospital was examined. In all of these patients there was a slight reduction. In two instances urine of patients suffering with nephritis was mixed with the blood of a normal individual, and in both of these it inhibited the decomposition of hydrogen peroxide much more than normal urine used in the control experiment.

Briefly summarizing the above, we find that in nephritis the kidneys as well as the other organs including the blood show a decrease in their catalytic activity, while the acid urine from a patient with nephritis has a more marked inhibiting action upon catalase than normal urine. It was thought that the inhibition noted when acid nephritic urine was used might depend upon the reaction of the urine. These results led us to the conclusion that the kidney in nephritis secretes into the body substances which manifest themselves in the above described decrease of the catalytic activity of the blood and organs.

If this hypothesis proves to be true the determination of the catalytic activity of the blood should be an easy clinical method of differentiating nephritis from analogous clinical conditions as eclampsia, etc., where there is no decrease in the catalytic activity of the blood.

With these facts in mind this work was undertaken to determine, if possible, the following:

1. Is the urine of a nephritic more inhibiting to catalase than

normal urine, or is the inhibiting action of the urine dependent upon its reaction?

2. Is the catalytic activity of the blood of a normal individual constant?

3. Does the retention of the products of metabolism normally excreted by the kidneys in the urine have any influence on the catalytic activity of the blood and organs?

4. Does the kidney in nephritis secrete substances into the blood which inhibit catalytic activity or is the decreased catalytic activity of the blood and tissues in nephritis due to the inability of the kidney to excrete anti-catalytic substances normally present in the blood?

5. What is the significance of the deceased catalytic activity of the blood and organs in nephritis?

Urine itself has no catalytic action, so to determine the influence it may have on an active organic catalyzer, as for instance normal blood, it is necessary only to mix them together. In this work the blood of a single rabbit furnished the catalase. The method employed was as follows: a graduated cylinder with capacity of ten cubic centimeters was filled to the mark indicating nine cubic centimeters with distilled water. Then the ear vein of the rabbit was punctured and one cubic centimeter of blood allowed to run in the graduate, giving a dilution of 1 to 10 of blood in distilled water. It is necessary to further dilute this solution because in all of the experiments only five cubic centimeters of hydrogen peroxide were used and this could be decomposed by five cubic centimeters of a 1 to 10 dilution in a few seconds. It was desirable to have the blood and hydrogen peroxide in such proportion that the five cubic centimeters of hydrogen peroxide would be decomposed during the first 15 to 30 seconds. Therefore, dilutions were made from this 1 to 10 stock dilution of the following strengths:

Dilution.	Seconds.							
	15	30	45	60	75	90	105	120
1–50	48	49.6	50.8	51	51.2			
1–100	30.4	39.8	45.6	49.4	50.6	50.8	51.2	51.2
1–200	17.2	22.4	27.6	31.6	35	38.2	40.8	43
1–400	9.8	12	14.2	15.8	17.6	19	20.6	22.6

1 to 50, 1 to 100, 1 to 200 and 1 to 400. These were tested by the same method described in the previous article.

The above table shows that a dilution of 1 to 50 yields a maximum evolution of gas from the five cubic centimeters of hydrogen peroxide in the first fifteen seconds, and the fact that up to the end of the first minute there is only a very insignificant further reaction shows that practically no undecomposed hydrogen peroxide is left. It was thought, therefore, that a 1 to 50 dilution would be a delicate indicator of any inhibitory action. Urines were obtained from several normal individuals and from several patients with nephritis in the wards. Toluene was used as a preservative. A dilution of 1 to 50 was made as follows: a solution of rabbit's blood diluted 1 to 10 was obtained as above and 1 c.c. of this solution was added to 4 c.c. of urine. The total acidity of the urine was determined by Folin's method, *i. e.*, precipitate 10 c.c. of urine with saturated solution of potassium oxalate, then titrate against $N/20$ sodium hydroxide using phenolphthaleine as indicator. The total acidity is recorded in terms of cubic centimeters of $N/20$ sodium hydroxide.

TABLE I.

Case No. I.—Normal Individual.

	Total Acidity in N/20 NaOH.	Seconds.							
		15	30	45	60	75	90	105	120
June 29, '08	5.0 c.c.	26	33.6	37	40.2	44.6	44.6	45.8	46.2
" 29, '08	6.4 "	6	11.6	15.2	19.4	23.2	26.2	30.4	34
" 30, '08	16.1 "	4.8	7	10.2	13	15.2	18.4	21	23.8
July 1, '08	14.6 "	5.8	8.2	10.6	13	16.4	19.2	22	24.4

Case No. II.—Chronic Diffuse Nephritis.

	Total Acidity in N/20 NaOH.	Seconds.							
		15	30	45	60	75	90	105	120
June 27, '08	8.8 c.c.	6.2	15.6	20.6	27.2	32	36.2	39.2	40.4
" 29, '08	5.2 "	14.1	27	39	47	49.8	50.6	50.6	50.8
" 30, '08	6.3 "	12.4	25.2	35.6	43.2	46.8	48.4	49.2	49.6
July 1, '08	9.6 "	10.2	23.8	31.4	42.6	45.2	47.6	49	49.6

The curves (Fig. 1) show the relation of the acidity of the urine to the catalytic activity of the blood.

These two cases show that the presence of nephritis alone is

not the important factor in determining the inhibiting power of urine on the catalytic action of blood. On the other hand, they show that the curve of the catalytic activity of the blood-urine

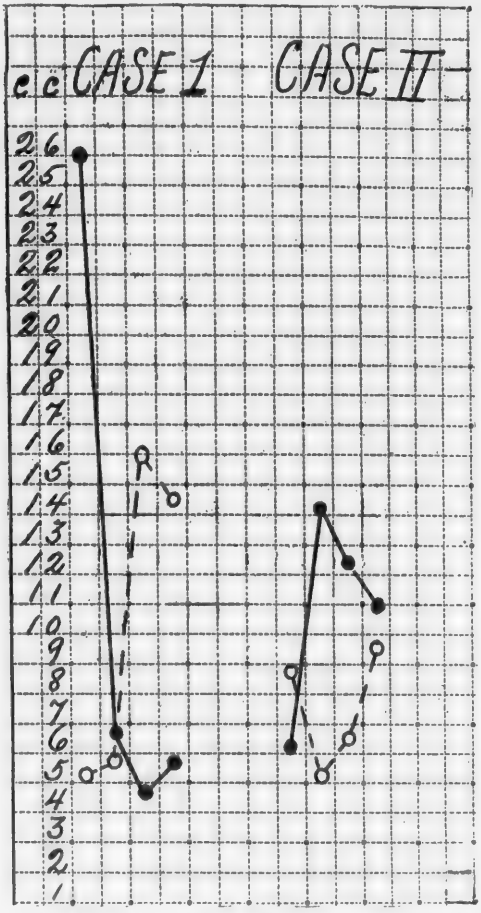

FIG. 1.

mixture varies inversely with the curve of the total acidity of the urine. It seemed, therefore, of the utmost importance to eliminate the question of acidity and to test the action of urine that had been

neutralized. The following tables will show duplicate readings, the unneutralized urines being above, the neutralized ones being below and designated by the letter *N*.

It seems quite definitely established by the above experiments that the most important factor which determines the inhibiting power of any urine is its acidity.[3] Likewise, when alkaline urine

Case No. I.—Normal Individual.

	Total Acidity in N/20 NaOH.	Seconds.							
		15	30	45	60	75	90	105	120
	13.5 c.c.	5	7.6	10	12.8	15.4	18	20.6	23
		N 40.2	47.6	49.2	50				
July 3, '08	11.8 "	8.4	12.4	16	20	23.8	27.6	31.2	34
		N 38	43	49	50.4				
" 4, '08	17.6 "	6	12.4	17.4	22.2	26.8	31	34	38.4
		N 34	46.6	49.8	50.2				
" 8, '08	6.2 "	9.4	13.6	17.2	[illegible]	24.2	27.6	31.8	37
		N 45	50.4						

Case No. II.—Normal Individual.

	Total Acidity in N/20 NaOH.	Seconds.							
		15	30	45	60	75	90	105	120
July 2, '08	12.8 c.c.	28	41.6	49	49.6	50.2	50.2	50.4	50.4
		N 44	49	50	51				
" 4, '08	4.6 "	42.4	50.4						
		N 48	50.4						
" 6, '08	5 "	27.6	46	50.2					
		N 36.4	49	57					

Case No. III.—Carcinoma of Stomach.

	Total Acidity in N/20 NaOH.	Seconds.							
		15	30	45	60	75	90	105	120
July 10, '08	6.7 c.c.	12.4	21	29.6	38.4	46	50		
		N 40.4	45	50					
" 11, '08	5.7 "	18.6	29	37.4	44.4	48.6	50.2		
		N 50.6							
" 14, '08	5 "	15.8	27.2	39	49.4	54			
		N 52							
" 15, '08	45 "	17.8	27.6	36.8	45.2	50	52.6		
		N 52							

[3] It is interesting to note the results of Loevenhart (*Amer. Jour. of Physiol.* 1905, xiii, 171) in this connection. He found that when unneutralized hydrogen peroxide is used, pancreas extracts markedly accelerate the decomposition of hydrogen peroxide by liver extract and that this acceleration is not effected when neutralized hydrogen peroxide is used.

Case No. IV.—Chronic Diffuse Nephritis.

	Total Acidity in N/20 NaOH.	Seconds.							
		15	30	45	60	75	90	105	120
July 4, '08	7.6 c.c.	16.4 N 44.6	27 48	36.4	43	46.4	49		
" 6, '08	7.2 "	11 N 34	17.6 40.2	27 44.2	32 48	36.2	40.6	44.4	11.8
" 15, '08	6.0 "	12 N 50.6	25	33	41	46.2	49.6	57.8	52
" 17, '08	5.8 "	6 N 58.2	15.2	23.8	32.6	40.4	45	49.6	51.8

Case No. V.—Chronic Diffuse Nephritis.

	Total Acidity in N/20 NaOH.	Seconds.							
		15	30	45	60	75	90	105	120
July 5, '08	2.4 c.c.	25 N 45	35 49	41 50	45.6 50.2	49.4	50	50.2	50.4
" 4, '08	8 "	33.8 N 45.4	45.6 50.4	50.4					
" 6, '08	3 "	27 N 43.6	37.6 49.4	45.4	48				

Case No. VI.—Chronic Nephritis.

	Total Acidity in N/20 NaOH.	Seconds.							
		15	30	45	60	75	90	105	120
July 4, '08	1.9 c.c.	36.4 N 41.8	43 46	47 50					
" 6, '08	6 "	27 N 48	42 57	57					

is examined it is found that the inhibition it exerts on the catalytic activity of the blood is similar to that observed with acid urine, that is, the greater the alkalinity the greater the inhibition. These experiments justify us in concluding that neutral urine, whether taken from a normal individual or a nephritic patient, has practically no inhibiting powers and that the slight inhibition which has been obtained in some of the determinations has been due to error in neutralization. This is the more likely since the color of the urines varied considerably from day to day, in this way interfering with the acidity determinations, and even slight variations from the neu-

tral point considerably influence the inhibiting power of the urine (See Case VI).

Is the Catalytic Action of the Blood of a Single Individual Constant?—Before it is possible to determine variations in the catalytic activity of the blood of rabbits resulting from experimental conditions, it becomes necessary to determine what the daily variations in the blood of a normal animal are, and as a secondary consideration to note whether there are any variations in different animals of the same species. In the work which is to follow, the blood has been used in such dilution that the decomposition of the hydrogen peroxide is not entirely finished within two minutes. This is desirable in order to make the velocity of the reaction more conspicuous. By referring to the table of dilutions (page 4) it will be seen that a dilution of 1 to 200 answers this purpose. In all of the following experiments this dilution was used. The animals were kept over a definite period of time, usually a week, and each day their blood was tested. It will be sufficient then to refer to any of these cases and note that the catalytic action of the blood of a normal rabbit is constant; for the variations noted, fall within the error of the method.

On the other hand the activity of the blood of different rabbits may vary within relatively wide limits. Just what this is dependent upon has not been determined, but it is significant that rabbits of the same litter and of the same grade of development have been much more constant than those where there was a visible difference in size and age. The younger and more poorly nourished ones have the least catalytic activity in their blood, while the older and better nourished ones seemed to have the highest activity.

In many of the following experiments it was necessary to anæsthetize the animal. It was important, therefore, to determine what influence prolonged anæsthesia had on the catalytic activity of the blood. Accordingly two rabbits were anæsthetized with ether for half an hour. No change was found in the catalytic activity of the blood as a consequence of the prolonged use of ether.

Does Retention of the Products of Metabolism Normally Excreted in the Urine Have any Influence on the Catalytic Action of

the Blood and of the Organs?—Since it has been shown that the catalytic activity of the tissues of an individual who has succumbed to nephritis as well as his blood during life has a decreased catalytic activity, it has seemed that by entirely preventing the function of the kidney as an excretory organ some similar changes may result. Therefore, after the catalytic activity of the blood of two animals had been found constant for several days and their urine free of all pathological constituents, their ureters were doubly ligated and cut between the ligatures. They recovered rapidly from the anæsthesia and the operation.

No. i lived seventy-eight hours and died under uræmic symptoms. At autopsy the operative incision was healing *per primam* and there was no sign of infection. The peritoneal cavity contained a slight excess of greenish fluid. Both *ureters* were found cut and ligated, the proximal ends retracted and their walls extremely congested. The *kidneys* were swollen and pale and on incision exuded a considerable amount of urinous fluid. The pelves were not dilated but contained a quantity of granular material which was microscopically composed of renal epithelial cells, hyaline, granular and epithelial casts. The cortex was swollen and showed yellowish areas of degeneration throughout. Microscopically the tubules throughout the kidney were dilated and contained within their lumina desquamated epithelial cells, casts and detritus. The epithelial lining was everywhere swollen and showed extensive parenchymatous, granular and vacuolar degenerations. The glomeruli were prominent due to the congestion. The *liver* showed an extensive vacuolar degeneration which began about the hepatic veins and extended almost to the portal spaces. No. xi lived seventy hours. The autopsy findings were practically identical with those of No. i.

At the end of every autopsy five grams of kidney, liver and lung were carefully weighed and 20 per cent. aqueous extracts made by grinding each organ in a mortar with finely divided washed-white sand. At least three extractions were made in every case, the emulsion being strained through coarse toweling by pressure. One cubic centimeter of the 20 per cent. cloudy extract thus obtained was placed in a salt-mouth bottle with 4 c.c. of distilled water; a small vial containing 5 c.c. of neutralized hydrogen peroxide were then placed in the larger bottle which was connected with a gas burrette and the catalytic activity of the organ determined. These results will be found in each case beneath the table of the catalytic activity of the blood.

Rabbit No. I.

	Seconds.							
	15	30	45	60	75	90	105	120
June 29	17.2	22.4	27.6	31.6	35	38.2	40.8	43
July 1	17.2	22.4	26.6	30	33.6	37	39.4	41.6
" 2	17.6	21.4	29.6	34	36	39.4	41.4	44
" 3	17.4	23.6	28	32	35.6	39	41.4	43.8
" 5	16.8	24.4	28.8	34	36.2	38.8	41	43.4
Operated July 7, 10.30 A. M.								
July 7, 3 P. M.	18.2	30.2	35	38.8	41.2	42.6	44.8	47
" 8	17.8	22.	25	28.8	33.2	35.6	38	40.4
" 9	14.6	18.6	21.6	24.8	27.8	30	33.2	36.4
" 10	12.8	17.4	21	24.4	27.2	30	32.4	35
Lung.	7.4	9.8	11.2	13	14.4	15.8	17.2	18.4
Liver.	20.4	27.2	33.4	36.2	41.6	45.4	48.4	50.6
Kidney.	10.4	14.8	17.4	19.8	22.4	25.2	27.4	29.4

Rabbit No. XI.

	Seconds.							
	15	30	45	60	75	90	105	120
July 21	17.6	22.4	26.8	30.2	33.4	36.8	40.6	42
" 22	17.2	23	26.8	31.2	35	37.4	41.2	42.4
" 23	17.8	23.4	27	30.2	34.6	38	40.8	42.8
" 24	17.6	22.6	26.8	30.8	34.2	37.2	41.4	43
" 25	17	23	27.6	31	33.4	36.4	39.2	40.8
" 26	17.4	23.2	27.2	31.2	35	37.2	40.4	40.2
Operated July 28, 1 P. M.								
July 29	17.2	22	25.2	29.4	32.6	35.8	38.6	41.4
" 30	14.8	18.8	22.2	25	28.4	31	34.4	35.8
" 31	9	11.4	13.6	15.8	18	20.4	22.4	21.6
Lung	8	10	11.8	13.6	15	16.2	17.4	18.6
Liver	14	19.8	24.6	29.6	33.8	38	41.8	45
Kidneys	15	20.2	25.2	28.4	32.2	35.4	39	41

The above tables show clearly that while the catalytic activity of the blood was constant before the ureters were tied, following the operation it rapidly declined until death. Furthermore, both the liver and kidneys show a decided decrease in their catalytic activity as is readily seen by comparing the above results with those obtained from the organs of a normal rabbit.

	Seconds.							
	15	30	45	60	75	90	105	min
Kidney	33	43	48.6	52.4	54	54.2	54.2	54.2
Liver	28.6	37.8	44.2	49.4	52.4	54	54	54.8

Figs. 2 and 3 are charts of the catalytic activity of the blood from day to day in which, however, only the readings of the first fifteen seconds are recorded.

Does the Kidney Excrete any Catalytic Inhibiting Substance, or is the Decreased Catalytic Activity of the Blood in Nephritis Due to the Inability of the Kidney to Excrete Anti-catalytic Substance Normally in the Blood?—In the last series of experiments although the excretory function of the kidneys was eliminated, the possibility of their influencing the catalytic activity of the blood by an internal secretion or by any process which their presence might effect could not be eliminated. Therefore, it was determined to try what effect the extirpation of the kidneys would have on the catalytic activity

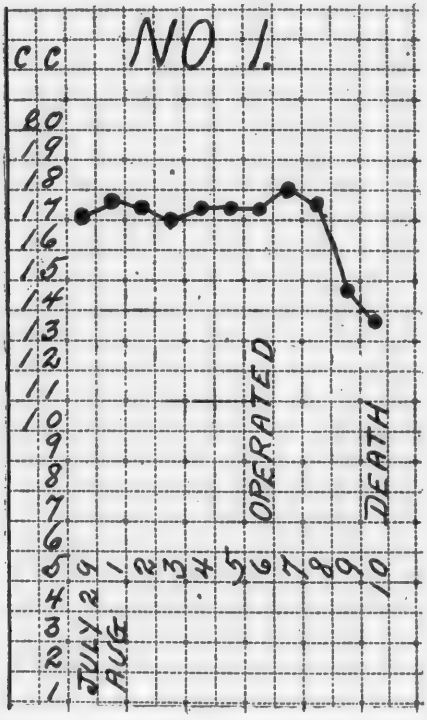

FIG. 2.

of the blood. This experiment was performed in the two following ways:

1. Bilateral simultaneous nephrectomy.

2. Unilateral nephrectomy followed, after the lapse of an interval of time sufficient for the animal to recover completely, by extirpation of the second kidney.

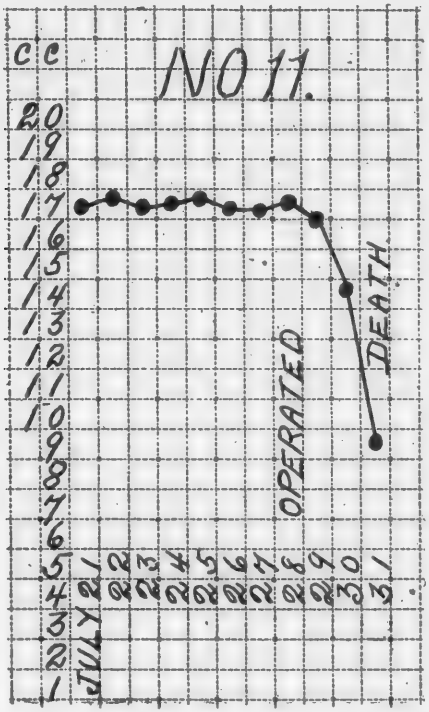

FIG. 3.

The operation was done under ether anæsthesia through the midline. This operation was preferred since by this route the kidneys could be easily reached and the operation quickly finished.

Rabbit No. 26.—The animal recovered promptly from the operation and seemed very comfortable for the first thirty-six hours; then it showed some respiratory distress, and bleeding became more and more difficult. After forty-

eight hours it could not be bled from the ear and the experiment was concluded by anæsthetizing the animal, exposing its heart and using the heart's blood for a final determination. At autopsy the incision was found healing well and there was no sign of infection about the ligated vessel or ureters.

The following table shows that while the catalytic activity of the blood was quite constant until the time of operation as early as three hours after there was a perceptible fall in the activity which continued throughout the life of the animal.

Blood—Dilution, 1 to 200.

	Seconds.							
	15	30	45	60	75	90	105	120
Aug. 20	21.4	27.6	32.8	37.2	41	44.4	47.4	49.6
" 21	21	27.6	32.6	36.8	40.6	44.2	46.4	48.6
" 22	20.6	25.8	30	34	37.6	40.4	43.4	45.4
" 23, 1 P. M.	21	26	31	35.8	39.6	43	45.8	48.2
" 23, Operated 3 P. M.								
" 23, 6 P. M.	19.8	25.6	29.8	34	39	41.8	44	46.2
" 23, 11 "	18	24.4	29.4	33	37.6	40.2	43	44.8
" 24, 9 A. M.	17.6	23.4	28	31.4	35	38	41	43.8
" 24, 2 P. M.	17.2	21.4	25.4	28.6	31.4	34.4	37.2	39.8
" 24, 6 "	17.4	22	26	29.2	32	35	37	39.2
" 24, 10 "	16.6	20.4	23.8	26.4	29.4	33	36.2	38
" 25, 7 A. M.	16	19.8	23.4	24.8	27.4	30	33.2	36.2
" 25, 11 "	15	19.4	21.8	24.6	26.6	29	31.2	33
	Heart's Blood.							
" 25, 2 P. M.	14	17.8	20	23.4	25	27.4	29.6	31.4

The curve of the catalytic activity (Fig. 4) as determined in the first fifteen seconds is quite typical of the entire process.

It is only necessary to compare the above table and curve with that of Rabbit No. 1 when it is found that they agree in every detail. Following bilateral extirpation of the kidneys, or bilateral ligation of ureters the catalytic activity of the blood immediately begins to decline and continues to do so until death. These results make it improbable that an internal secretion of the kidneys has any influence on the catalytic activity of the blood. Neither does it seem that any function they may subserve aside from their function as excretory organs enters into consideration in this regard.

Rabbit No. 18.—The animal was etherized and the left kidney extirpated through a left rectus incision. The animal recovered promptly from the operation. Microscopic examination of the kidney showed no pathological change. Following nephrectomy the catalase of the blood soon began to fall and with this the animal's condition became rather grave. Unfortunately in this case the urinary findings were not determined but in several similar cases where these were carefully watched, it was found that besides albumin and

casts the amount of urine became greatly diminished. In this case the course of the experiment showed that the other kidney was able to compensate for the loss of the extirpated kidney. On the other hand in Rabbit No. 10 in which one kidney was removed after an experimental nephritis followed by uræmia had been produced, the remaining kidney could not compensate for the sustained loss and the animal died. In this case the catalytic activity of the blood de-

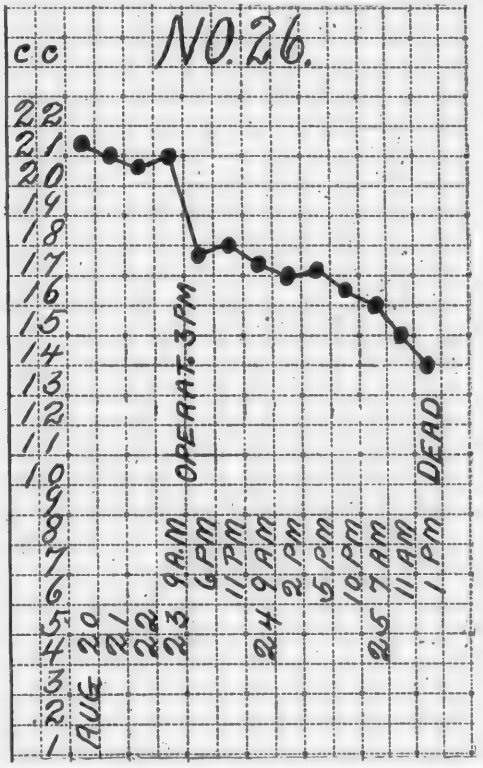

FIG. 4.

creased from the time of operation until death (see Fig. 13). However, after being quite low for several days the catalytic activity of this animal's blood (No. 18) again began to rise rapidly and assumed a constant level somewhat higher than that at which it had been previous to operation. At present I cannot venture an explanation of this occurrence. The same result was obtained in several nephrectomized animals as well as in animals in which an experimental nephritis had been produced.

After the catalytic activity of the blood (No. 18) had become constant, the right kidney was removed through a right rectus incision. This showed microscopically a slight dilatation of the uriniferous tubules and some swelling and vacuolar degeneration of their lining membrane. The glomeruli were enlarged and an occasional cast was seen. The animal lived fifty-five hours after the second operation. At autopsy nothing abnormal was made out. There was no sign of inflammatory reaction about either operative wound.

The following table is a record of the catalytic activity of the blood during the experiment.

Rabbit No. 18.

Blood—Dilution, 1 to 200.

	Seconds.							
	15	30	45	60	75	90	105	120
August 3	17.2	23.2	27.6	31.6	35	38.2	40.4	42.8
" 4	17.4	22.4	27.2	31.2	34.6	38	41	43.4
" 5	17	22.6	27	31	34.6	37.6	40.2	42.4
" 6	18	23	27.2	29.6	33	37.2	40.6	43.4
" 7	17.8	25.6	27.8	31.8	34.2	38	40.8	43.6
" 8	18.6	24.8	29.4	32.8	34.6	39.6	42	44
" 8, Operation—Left nephrectomy, 2 P. M.								
" 9	20	25.6	30.4	34.2	38.4	42.6	47.8	50
" 10	19.2	24.8	28.4	31.8	36	39.4	42.6	44.4
" 11	17.4	23.2	27	31	34.8	37	40.2	43
" 12	15.8	20.6	25.8	29.4	32.8	35.4	39	41.2
" 13	13.8	18.4	22.6	26.8	30	33.6	36	39.8
" 14	10.8	15.2	19.4	26	29	31.6	35.4	39
" 15	15.6	20.4	26	29.8	33.4	37	40.4	47.8
" 16	19.6	26.4	32	37	41	44.6	46.8	49
" 17	19.2	25	29.8	34.2	37.6	41.6	44.8	47.6
" 18	19.6	25.4	31.8	36.8	41	44.8	47.4	49.6
" 19	19.	23.8	28.2	32.8	36.2	39.4	42.4	45
" 20, P. M.	19.6	25	29.6	34.8	39.4	42.6	45.4	48
" 20, Operation—Right nephrectomy, 3.30 P. M.								
" 20, 5 P. M.	19.4	25.6	31.4	35.8	39.8	43.4	48.8	48
" 20, 10 "	18.2	23.4	27.8	32.8	37.6	39.8	43.8	45.2
" 21, 7 A. M.	21.6	27.4	30.4	33.2	36.4	39.3	41.2	43.6
" 21, 10 "	17.8	22.6	26.4	29.8	32.8	35.6	38.4	41
" 21, 1 P. M.	17.2	22	25.6	29.4	31.4	36	39.4	41.6
" 21, 5 "	17.2	22.6	25.4	29.6	33.4	36.4	39.6	41.8
" 21, 11 "	17.2	22	26	30.2	33.6	34	36.6	39.2
" 22, 9 A. M.	16	20.4	24.2	27.4	30.8	30.6	33.2	35.4
" 22, 1 P. M.	14.2	18.6	21.8	24.6	27.8	30	33.2	35.4
" 22, 2 "	13.8	18	20.6	24.8	27.2	21.4	32.4	34.8
" 22, 4 "	13.2	18	21.8	25.6	27.6	31.4	32.2	35
" 22, 6 "	13.4	17.4	21	24.6	27	30.4	33	35
" 22, 8 "	12.6	16.2	19.8	22.6	25.4	27.6	30	32
" 22, 10.30 P. M.	12.6	16.8	20	23.6	26.8	28	30.2	32
" 22, 11 "	Dead							

It will be observed that the end result is the same when both kidneys are removed at once, when they are removed separately in two stages, and when the ureters are ligated. At present it may

be assumed that the decline in the catalytic activity of the blood is a direct result of the retention of metabolic products normally excreted by the kidneys in the urine. It seems that as far as the catalytic activity of the blood is concerned only this function of the kidneys enters into consideration.

It remains now to see whether the retention of urine in the body

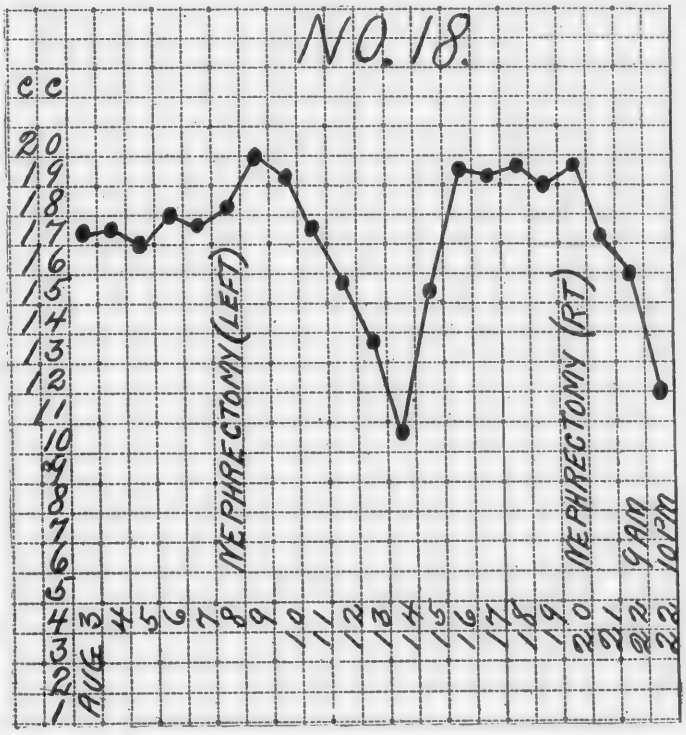

FIG. 5.

after it has been excreted by the kidneys has any similar influence. First, however, the fall following the primary nephrectomy in No. 18 is worthy of note. There seems little doubt that in this animal the crucial point had been reached and had the catalytic activity of its blood not soon begun to rise the low action could probably

have been taken as a serious prognostic sign. This was shown in No. x (Fig. 13) where the remaining kidney could not compensate since it was diseased and together with the steady fall of the catalase the symptoms became more and more grave and the animal died. It seems, therefore, that this reaction may prove of significance in post-operative cases where it is necessary to know the sufficiency of the remaining kidney.

What is the Effect on the Catalytic Activity of the Blood if the Kidneys are Allowed to Excrete Urine, but the Urine is Retained in the Body?—The catalytic activity of the blood is greatly diminished if no urine is excreted, no matter whether the kidneys are left in the body or not. It seems desirable, therefore, to conduct experiments with a view of determining the catalytic activity of the blood in such cases where the kidneys are able to discharge urine, while at the same time the urine is retained in the body. In the three following rabbits, Nos. 15, 16, 17, the ureters were cut, the distal ends being tied while the proximal ends were allowed to drain into the peritoneal cavity.

Rabbit No. 15.

	Seconds.							
	15	30	45	60	75	90	105	120
July 30	17	21.4	25.	29.4	33.6	37	40.2	42.6
" 31	16	20.4	24	27.4	30.6	33.8	36.6	39.2
Aug. 2	16.6	21.4	25.6	28.6	32	35.4	38	40.8
" 3	16.2	20.4	28	31.4	34.4	37.6	40.2	
" 3, Operation								
" 4	18.4	25.4	30.6	35.4	39	42.8	45.6	48
" 5	17	22.2	26.8	31	38.2	38.4	40.8	43
" 6	16.4	20.6	24	27.2	30.2	33.4	33.8	38.4

After operation the animal seemed quite well for the first thirty-six hours. Its abdomen became slightly distended, and its distention increased gradually. There was a distinct movable dullness in the abdominal cavity. At midnight on August 5 he was found with his head on the side of the table breathing laboriously, and had a very feeble pulse. He died at 12.10.

The autopsy showed the scar in the anterior abdominal wall healing *per primam*. The abdomen was markedly distended. The abdominal cavity contained a large amount, about 300 c.c., of a cloudy, yelowish fluid. This showed microscopically many epithelial cells of renal type, a few large, swollen squamous cells and a few white blood cells and flakes of fibrin. The peritoneal surfaces over the large intestine were everywhere injected. The left ureter was distended

and injected, due to inflammatory adhesions which constricted its free end. The distal end was ligated. Along the course of the left ureter, in the retroperitoneal tissue there was an extensive infiltration of fluid similar to that found in the abdominal cavity.

The *left kidney* was very much enlarged and presented a curious appearance, being mottled, large reddish-black areas alternating with pale brown areas. The cortex was swollen, and grayish yellow opaque areas were scattered

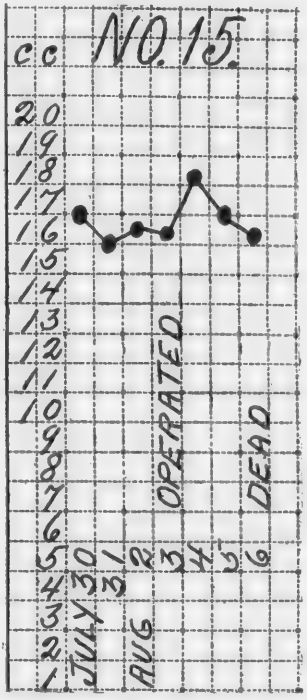

FIG. 6.

through it. The pyramids were pale except where the areas of hæmorrhagic infiltration had invaded it. The pelvis was slightly dilated and contained a golden yellow material which on microscopic examination was composed of many cells, among which numerous red blood cells and renal epithelial cells were found. The *right ureter* was normal in position and appearance. The proximal end was patent. The distal end was closed by a ligature. The *right kidney* was smaller than the left, but had the same general appearance externally. On section it resembled the left except that there were no yellowish gray areas of degeneration found and the pelvis was free of cellular detritus.

Microscopically the kidneys showed their tubules to be dilated with the epithelium swollen, pale-staining, granular, and here and there desquamated. Considerable amounts of extravasated blood were found both in the glomeruli and in the tubules. (The appearance of the epithelial lining cells of both the uriniferous and collecting tubules full of fluid, as they apparently were, impressed one as if they were reabsorbing the urine from the tubules.)

The twenty per cent. aqueous extracts of the organs exhibited the following catalytic activity:

	Seconds.							
	15	30	45	60	75	90	105	120
Kidney	17.4	23.4	28.6	33.4	37.8	41	45	47
Liver	17.2	25	30.8	35.6	39.6	44	47	49.4
Lung	6.8	8.2	9.8	11.2	17.6	14	15.2	16

This experiment is unsatisfactory in as much as the left ureter was occluded. It is, however, interesting to note that there was a primary rise and then, as the one ureter was occluded, a slight fall. This will be clear as soon as the remaining cases of this series are studied.

Rabbit No. 16.

	Seconds.							
	15	30	45	60	75	90	105	120
July 30	20.2	26.8	31.2	35.2	38.6	42.8	44.8	48
" 31	21.8	28.8	33	37.4	41.4	44.8	47.6	50
Aug. 2	22.6	28.8	33.8	38.4	42.4	45.6	48.2	51
" 3	22	28.8	33.6	38.4	42	45	48.4	50.6
" 4	22.6	29.6	35.2	40	44.4	47.8	50.4	52
" 5	22.2	29.6	35.4	40.6	44.6	48	50.4	51.6
" 6	22.6	29.8	35.6	40.2	44	47.6	50	52
" 6, Operation 4 P. M.								
" 7, 9 A. M.	33.6	41.8	47.6	50				
" 7, 12 M.	34.6	44.6	50.4	53.8				
" 7, 4 P. M.	37.6	47.4	51.6	53.6				
" 8, 7 A. M.	36.8	46.4	50.4	53				
" 8, 4 P. M.	31.6	40.8	45.4	50	52.2			
" 9, 1 A. M.	25.8	33.4	39	44	48	51		

This animal died fifty-seven hours after operation. The abdomen had become distended. There was marked movable dullness and the kidneys seemed somewhat enlarged. At autopsy the abdominal cavity contained a large quantity of cloudy yellowish fluid which coagulated on standing. The peritoneal surfaces were everywhere covered by a very extensive fresh, fibrinous exudate. The incision, however, was healing by first intention. The ureters were cut near the entrance to the bladder, but the proximal ends were patent and their walls normal in appearance; the distal ends were tied. The kidneys were swollen

and of a grayish color. The cortex was swollen and pale. The pyramids were slightly congested. The pelves were quite normal. Microscopically the kidney tubules were slightly dilated and their epithelium swollen slightly. The liver showed some parenchymatous swelling of its cells.

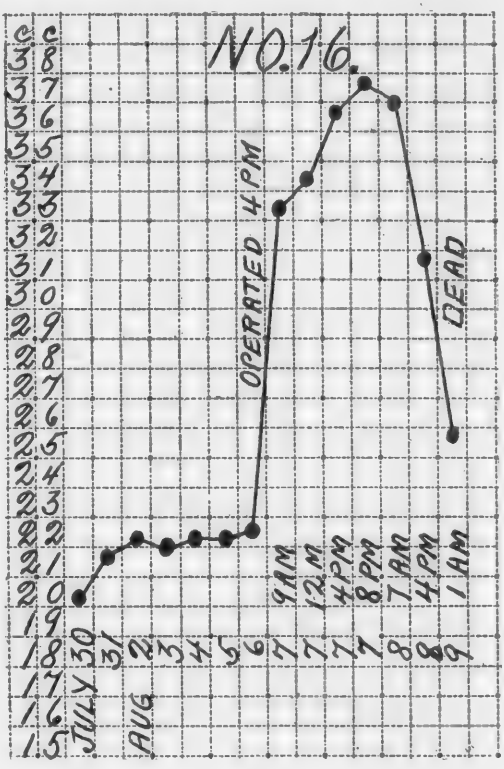

FIG. 7.

Twenty per cent. aqueous extracts of the organs had the following catalytic activity.

	Seconds.							
	15	30	45	60	75	90	105	120
Kidney	28.8	38	45.8	51	54			
Liver	20.8	28.4	34.6	40	44.8	47.6	50	52.4
Lung	7.6	9.4	11.2	12.8	etc.			

It will be noted that in this case there was a marked rise in the catalytic activity of the blood and no decrease in the activity of the tissues, despite the fact that the animal had not voided urine for fifty-seven hours, and there was an extensive peritonitis following the drainage of the urine into the peritoneal cavity.

Rabbit No. 17.

	Seconds.							
	15	30	45	60	75	90	105	120
Aug. 1	21.6	27.4	32	36	39.8	43.2	46.4	48.8
" 2	20	25.4	30	33.8	37.8	41.8	44.2	46.4
" 3	19.6	25.6	29.8	34.8	37.2	40.8	43.4	46
" 4	20	25.2	30.4	34.8	38.6	40.4	45	47.6
" 5	20.4	26.8	31.6	35.6	39.4	43.2	46.2	48
" 6	19.2	25.2	30.4	34.6	38.8	43	45.8	48
" 7	19.6	25.8	31.4	36	39.6	42.8	45.4	47.6
" 8	20	26.2	30.2	33.8	37.6	41.6	44.4	46.2
" 8, Operated 2 P. M.								
" 8, P. M.	19.4	25.8	30.4	34.6	38.6	43.2	45.6	47.6
" 8, 11 A. M.	20	26	29.6	33.8	37.6	41.4	44.4	46.2
" 9, 5 P. M.	19.6	25	29.8	34	37.8	41.2	44.6	46
" 10, 9 A. M.	18.2	24	27 4	31.4	35	37.6	40.8	43.4
" 10, 4 P. M.	16.6	21.2	25.4	29.2	32.4	35.6	38	40
" 10, 10 P. M.	13.8	17.4	21	24.2	27.4	29	32.6	34.8

At 5 A. M., August 13, sixty-four hours after operation, the animal was found dead, quite cold and rigid. He had shown no untoward symptoms five hours previous, except a palpable enlargement of the kidneys and a constant steady decline in the catalytic activity of his blood. It seemed that somehow the ureters must have been occluded as the catalase here followed exactly the same curve as in Rabbit No. 11, and entirely different from that of No. 16, where a rise was noted.

At autopsy the abdomen contained no excess of fluid, but a few strands of fibrin were found on the peritoneal surfaces. The distal ends of the cut ureters were ligated, the proximal ends were much retracted and dilated, measuring above 1 cm. in diameter. Their free ends were closed by an inflammatory reaction. Their walls were markedly injected. The kidneys were mottled just as in Rabbit No. 15 except that microscopically the tubules were much more dilated and contained large quantities of serum and cellular detritus.

Twenty per cent cloudy extracts of the liver and kidney showed a marked reduction in the catalytic activity.

	Seconds.							
	15	30	45	60	75	90	105	120
Liver.	16.2	22.4	28	33.8	42.2	45.4	48	50
Kidney.	9.2	12.6	15.6	18.8	21.6	24.2	26.4	28.2

Summarizing this case, there was a very slight peritonitis, probably due to the primary extravasation of urine. This was most marked about the cut ends of the ureters and occluded them, causing bilateral hydro-ureter. As a consequence the catalytic activity of the blood and tissues assumed the same curve as when the ureters were ligated. (See Nos. i and xi, pages 14, 15.) On the

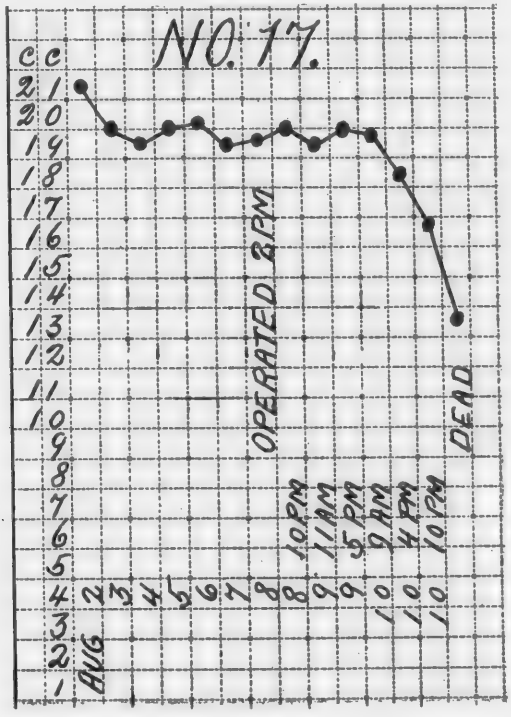

Fig. 8.

other hand in No. 15 where only one ureter was occluded there was a primary rise accompanying the peritonitis, but this rise was soon off-set by the retention of urine from the occluded kidney, while in No. 16 where both ureters were pouring urine into the peritoneal cavity there was a very marked rise. It seemed necessary to adopt a more satisfactory operation where there could be no occlusion of

the ureter and the urine would flow freely into the peritoneal cavity. Therefore, in Rabbit No. 23 instead of making a complete section of the ureters, these were nicked, small scissors inserted into their

Rabbit No. 23.

	Seconds.							
	15	30	45	60	75	90	105	120
Aug. 14	13.2	17	20.6	24.2	36.6	29.4	31.2	34
" 15	13.4	17.4	21.8	25.6	28.6	30.8	32	33.4
" 16	13.6	16.8	20.4	23	25.2	27.6	30	33.2
" 17	13.8	17.6	20.4	23.2	26	28.6	30.8	33
" 17, Operation, 3 P. M.								
" 17, 11 P. M.	20	25.6	29.8	33.4	37	40.2	43.2	46
" 18, 9 A. M.	17.4	21.8	25.4	28.2	31.4	34.2	37.2	39.6
" 18, 2 P. M.	16.6	20.6	24.8	28.4	32	34.8	37.4	39.4
" 18, 7.30 P. M.	16	20.8	25	28.8	33.8	34.8	37.4	39.2
" 18, 8 P. M., Heart's Blood	15.8	20.4	24.6	28	31.2	34.6	37	39.6

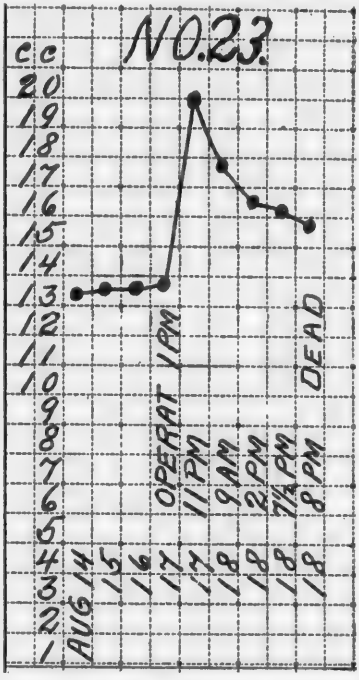

FIG. 9.

lumen and the wall cut on one side only for a distance of several centimeters. The ureter was then ligated distal to the false opening.

The animal's abdomen rapidly became distended and there was marked movable dullness. It died after thirty-one hours. At autopsy, there was a large excess of cloudy amber-colored fluid in the peritoneal cavity which coagulated on standing. The peritoneal surfaces were covered by a fibrinous exudate as in No. 16. There was no inflammatory reaction about the ureters and both drained freely into the abdominal cavity. The kidneys were slightly swollen, of a deep pinkish-brown color. The cortex was swollen, the striæ normal. The 20 per cent. cloudy aqueous extracts of the liver and kidneys showed no reduction of their catalytic activity.

	Seconds.							
	15	30	45	60	75	90	105	120
Kidney	28.2	39	45.6	50.2				
Liver	32.4	41.6	46.4	52				

This case corresponds in every detail to No. 16. Following drainage of the ureters into the peritoneal cavity there was an extensive peritonitis accompanied by a rise in the catalytic activity of the blood and organs.

Rabbit No. 24.—In order to minimize any possible reflex influences the section of the ureters might have on the renal function, the urethra was ligated, an elliptical piece excised from the fundus of the bladder, and the mucosa and serosa whipped together, leaving a wide opening between the bladder and the peritoneal cavity.

Blood—Dilution, 1 to 200.

	Seconds.							
	15	30	45	60	75	90	105	120
Aug. 16	17.8	25.4	29.4	34.6	37.2	40.4	42.8	45
" 17	18.8	23.8	28.4	32.4	36.2	39.6	42.4	44.4
" 18	18.4	22.8	28.6	32	35.4	39.2	41.8	43.8
" 18, Operation 3.30 P. M.								
" 18, 6 P. M.	19.8	25.6	30.4	34.6	38.6	42.2	44.6	46.8
" 18, 10.30 P. M.	22.6	29.4	33.8	38.2	41.8	45.2	47.6	49.6
" 19, 9 A. M.	22.6	29.4	33.8	38.4	42.6	46	48.4	51.4
" 19, 2 P. M.	24.8	32.8	38.8	44	48.6	51.6	54	55
" 19, 5 "	21.8	28.4	34.2	39.4	43.6	47.4	50	52
" 19, 10 "	22	30	34.8	40.2	44.8	48.2	51	53
" 20, 7 A. M.	21.2	29.4	32.6	37.8	41.6	44.2	48.2	50.4
" 20, 11 "	21.4	29.8	33	38	42	45	48.6	51
" 20, 12 M. Post-mortem.	21.4	28.8	33.4	37.6	41.2	45.2	48.4	50.6

After operation, the abdomen of the animal gradually became distended. The animal became stuporous and forty-five hours later was killed with ether since its blood pressure was so low that blood could no longer be obtained from the ear vessels. At autopsy the abdomen contained about 300 c.c. of turbid yellow fluid, containing microscopically many large mononuclear cells which

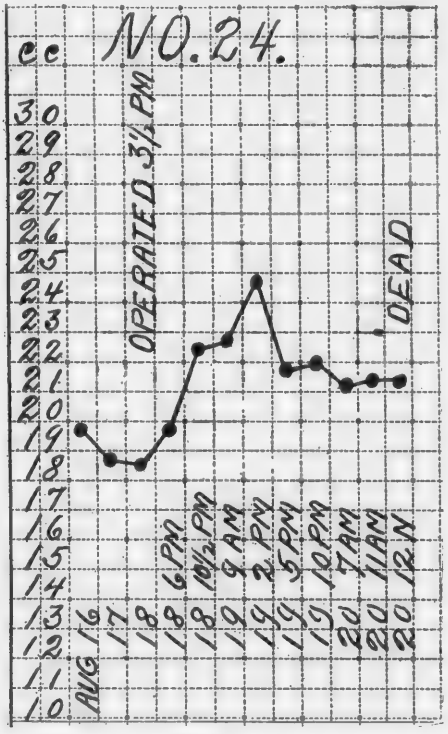

FIG. 10.

were actively amœboid, few polymorphonuclear and red blood cells. The peritoneal surfaces were covered by a dense fibrinous exudate. The *bladder* was collapsed but the artificial mouth was wide open. The *ureters* were normal, the *kidneys* slightly swollen, especially the cortex. Their catalytic activity was not reduced.

	Seconds.						
	15	30	45	60	75	90	105
Kidney	26	31.6	39.4	45.8	49.4	57.	

This case corresponds both in its pathological findings, and in the catalytic activity of the blood and tissues to Nos. 23 and 16.

From the above experiments we see that when the kidneys are allowed to excrete urine and the urine is drained into the peritoneal cavity, whether through the severed ureters or the bladder, there is an entirely different effect on the catalytic activity of the blood and tissues. When the ureters are tied or the kidneys extirpated there is a diminution of the catalase of the blood and the organs. When the kidneys discharge urine and the urine runs into the peritoneal cavity the organs suffer no change in their catalytic activity but there is a marked rise in the catalase of the blood accompanying the peritonitis. How can this rise be accounted for?

Rabbit No. 25.—After the catalytic activity of the rabbit's blood had been found constant for ten days, the animal was anæsthetized, the abdomen opened and several slices of potato saturated with a broth culture of staphylococcus aureus were placed in the peritoneal cavity. The wound was then closed.

Blood—Dilution, 1 to 200.

	Seconds.							
	15	30	45	60	75	90	105	120
August 20	19.2	23.6	28.4	32	35.6	39	42.2	44.4
" 21	19.8	24.4	28	31.2	34.8	37.4	40.6	43
" 22	20.2	25.6	29.4	33	35.8	39	42.4	44
" 24	20	25.8	29	32.8	35	38.4	41.4	44
" 26	20	26.2	29.8	32.6	35.6	38.2	40.6	43
" 27, 9 A. M.	20	25.8	29.2	32	34.8	37.2	39.6	42
" 27, 9.45 Operation								
" 27, 12 M.	23.6	29.2	33.6	36.2	38.6	41.2	43	45.2
" 27, 4 P. M.	28.2	36.6	41	44.6	47.4	49.4	51	51.8
" 27, 7.30 P. M.	25.4	31	35	38.8	41.8	44.4	46.6	48
" 27, 11 "	22.8	29	33.2	37	40.2	41.8	43	44.2
" 28, 9 A. M.	22.4	28.8	35.4	35.6	37.8	40.4	42.4	44
" 28, 12 M.	23	29.4	33.8	37.6	40	42.4	43.8	45

The animal recovered from the operation quickly but soon became drowsy and after thirty hours it was killed with ether. The abdominal cavity contained a slight excess of fluid, and the peritoneal surfaces were covered by a fibrino-purulent exudate which was most marked about the potato shavings. The organs showed parenchymatous degeneration.

In this case too a rise of the catalytic activity of the blood followed the experimental bacterial peritonitis just as in the cases of peritonitis following the drainage of urine into the peritoneal

cavity. The determination of the catalytic activity of the blood may aid the early clinical diagnosis of inflammatory processes of the peritoneum. The rise of the catalytic activity of the blood following the drainage of urine into the peritoneal cavity can be accounted for by the peritonitis which invariably followed, and, there-

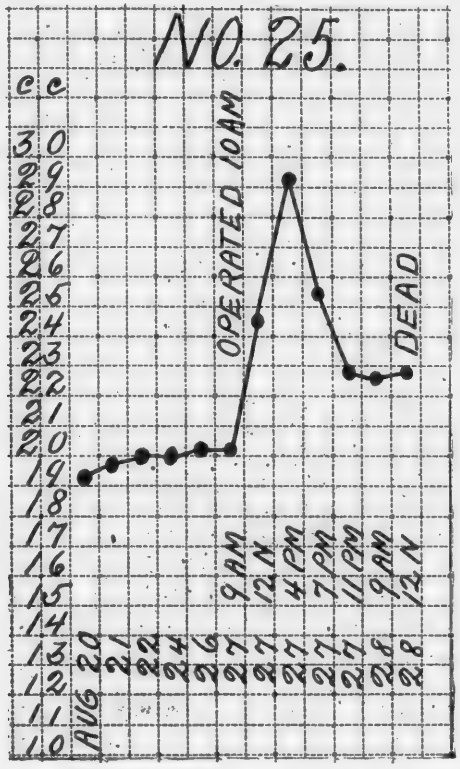

FIG. 11.

fore, there is no necessity to assume that the retention of urine in the body plays any rôle in this regard. When the ureters are ligated or the kidneys are extirpated, the catalytic activity of the blood and tissues decreases. These facts may perhaps best be explained by the assumption that the kidneys change substances circulating

in the blood into urinary products devoid of any influence on catalase. If the kidney cannot effect this change, these substances remain unaltered in the circulating blood and by their presence inhibit the catalytic activity of the blood and organs.

What is the Significance of the Decreased Catalytic Activity of the Blood and Tissues in Nephritis?—Experimental nephritis was produced in rabbits by the subcutaneous injection of uranium nitrate. In doses of fifteen minims, a one per cent. solution of uranium nitrate invariably killed a full grown rabbit; ten minims usually killed, but in some cases the animals survived. After five minims the animals always lived but in several instances showed marked uræmic symptoms. All the animals were kept in metabolism cages and the total quantity and specific gravity of their urine determined each day; the albumin and microscopic findings were recorded.

The cases may be divided into several groups:

1. Those that died after one injection.

2. Those that showed marked uræmic symptoms after one or two injections but recovered.

3. Those that lived over a period of months and had several injections, finally dying of nephritis.

Group 1.—Four animals receiving 10, 15, 15 and 20 ℿ of uranium nitrate, respectively, are included in this group. It will be sufficient to describe the following case as a typical one. These notes are abstracted from its history.

Rabbit No. 5. July 19.—On July 16 the animal received 20 ℿ of a one per cent. solution of uranium nitrate subcutaneously. For the past two days it has had diarrhea, and has eaten little; its urine has decreased in amount. This morning it was found lying in a cage with marked respiratory distress. During the morning it had many convulsions in which the whole body shook for several minutes. The convulsions were most marked in the head and extremities. The animal's condition constantly grew worse and at 2 P. M. it died.

At autopsy the abdomen was slightly distended. The peritoneal cavity contained a slight excess of yellowish clear fluid. The peritoneal surfaces, however, were smooth and glistening. The *kidneys* were slightly enlarged. Their surfaces were mottled, grayish-brown areas alternating with hæmorrhagic ones. The pelves were slightly distended. The cut surface showed a zone of hyperæmia about the periphery of the pyramids, and a grayish area of degeneration at the base of the cortex. Similar small, yellowish areas were found throughout the cortex. The bladder contained several cubic centimeters of clear amber-colored fluid in which many casts and a large amount of albumin were found.

Twenty per cent. aqueous extracts of the kidney and liver gave the following catalytic activity.

	Seconds.							
	15	30	45	60	75	90	105	120
Kidney	9.6	12.6	14.8	16.2	19.4	21.6	23.4	25.2
Liver	18.4	25.8	32.6	38.2	47.6	47.2	49.2	51.8

Microscopically the kidney showed extensive degeneration of the epithelial lining of the tubules. These cells were swollen, granular, vacuolated, and in places extensively desquamated, while in the lumen of the tubules, large amounts of detritus and granular and cellular casts were found. The glomeruli were slightly congested.

Blood—Dilution, 1 to 200.

	Seconds.							
	15	30	45	60	75	90	105	120
July 12	18	25.4	31.4	36	41	44	46.2	48
" 13	18.4	26	32	35.8	41.2	43	47.4	48.2
" 14	18	25.6	31	36.2	41.6	44	46.8	48.2
" 15	18.2	26	32	35.8	40	43.2	47	48.6
" 16, 20 ♏ 1% Uran. Nit.	18.2	25.2	30.6	33.8	40	43.2	47	48.6
" 17	16.8	22	26	29.8	33.6	36.4	39.8	43
" 18	12.4	17	20.2	23.6	27	29.8	32	35
" 19	10.4	14.6	17	19.6	22	24.3	27	29.8

Urinary Chart.

	Amount.	Sp. Gr.	Reaction.	Albumin.	Microscopical Examination.
July 12	130 c.c.	1014	Alk.	Neg.	No pathological elements.
" 13	125 "	1015	"	"	" " "
" 14	130 "	1013	"	"	" " "
" 15	140 "	1012	"	"	" " "
" 16	125 "	1014	"	"	" " "
" 17	75 "	1015	"	++	Casts: hyaline and granular. Renal epithelial cells.
" 18	80 "	1012	"	"	No pathological elements.
" 19	40 "	108	"	"	" " "

In Fig. 12 the solid dots represent the activity of the catalase of the blood in cubic centimeters of oxygen evolved during fifteen seconds (see figures on left side); the skeleton dots represent the total daily excretion of urine in cubic centimeters (see figures on right side of chart). The urinary findings are found at the bottom of the chart.

This is a typical case of the group of acute nephritides where there is a decreased urinary excretion with large amounts of albumin and casts and extensive renal degeneration. The above chart shows the curve of the catalytic activity of the blood, declining in exactly the same manner as the curve of urinary excretion. It will be

noted also that the catalytic activity of the tissues was greatly reduced. Practically the same results were obtained in this case as in those where there was a retention of urine, due to ligation of the ureters or bilateral nephrectomy.

Group 2.—In this group there are two animals that recovered from attacks of uræmia. One of these will be described.

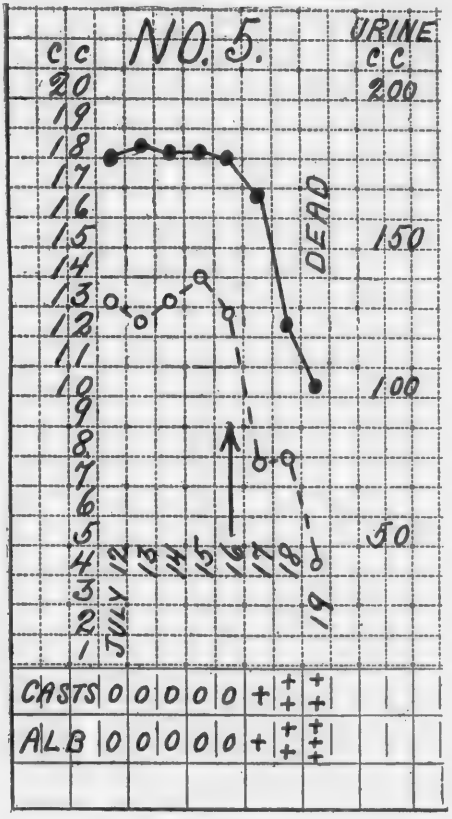

Fig. 12.

Rabbit No. 10.—The following notes were abstracted from the history. *August 10.*—After the urine had been found quite normal, and the catalytic activity of the blood almost constant for two weeks, the animal received, on July 29, 5 m. of one per cent. solution of uranium nitrate subcutaneously. Its

urinary excretion dropped gradually and on August 3 there was total suppression which continued for thirty-six hours. Its condition during this period was analogous to the condition of the other animals just before they had died consequent upon the injection of uranium nitrate. Its respirations were labored, and its pulse weak. The catalytic activity of its blood likewise decreased, falling lowest on the day when there was no excretion of urine. On the night of August 3 it began to void urine, and by noon of the next day it had excreted 220 c.c. Its condition immediately improved. The catalytic activity of its blood likewise increased rapidly, reaching normal on August 6, but the increase did not stop here. The level it assumed was, as in Rabbit No. 18, several points higher than it had been previous to the attack of uræmia. The urine showed a large amount of albumin and casts. But this condition gradually diminished until on August 8 the urine was again free of pathological elements. The animal's weight declined rapidly during the severe illness, but gradually rose again to its previous level. It seemed of the utmost importance to determine the condition of its kidneys at this stage. Therefore, its left kidney was removed this morning. It was slightly enlarged and of a very pale chestnut-brown color. Its surface was smooth and homogeneous in appearance. On section the cortex was somewhat swollen and of a dull grayish-brown. The striations were regular but obscured. The base of the cortex was of a much duller gray color while the pyramids had a peripheral pink zone.

Microscopically, the tubules were markedly dilated; their epithelial lining was swollen and in various stages of degeneration, showing vacuoles, coarse and fine granules, and a slight amount of desquamation. This was much more prominent in the uriniferous than in the collecting tubules. All the tubules contained granular detritus which in places took the form of casts. The glomeruli showed swelling of their lining cells and some congestion of the capillary tufts. There was no increase in fibrous tissue except in a few small areas where there was only a very slight excess.

The catalytic activity of this kidney was:

	Seconds.							
	15	30	45	60	75	90	105	120
Kidney	16.6	23.4	28.8	32.8	37.4	40	44.4	45.8

August 19, 1908.—Almost immediately after the operation the animal's condition became worse. The catalytic activity gradually dropped with only slight rises until August 19 when the animal died. The excretion of urine likewise dropped and on August 13 there was total suppression. This decrease, however, only lasted eighteen hours when it again began to void irregularly. The last twenty-four hours of its life, it voided only a very few cubic centimeters. It became progressively weaker and died this morning. At autopsy it was found much emaciated. The wound in the right rectus had suppurated slightly. The abdomen was normal. There was no inflammatory process about the area where the left kidney had been excised, but the ligated vessels were completely covered by serous membrane. The right kidney was swollen, very pale brown,

mottled by a few pinkish areas. On section the cortex was slightly thickened, the striations were obscured, and throughout the parenchyma yellowish-opaque areas of necrosis were seen. The pyramids were very pale. The catalytic activity of its tissues were determined as follows:

	Seconds.							
	15	30	45	60	75	90	105	120
Liver	10.6	14.6	17.8	21.2	24.4	26.8	28	31.2
Kidney	13.4	19.6	24.2	27.8	30.8	33.4	37.4	40

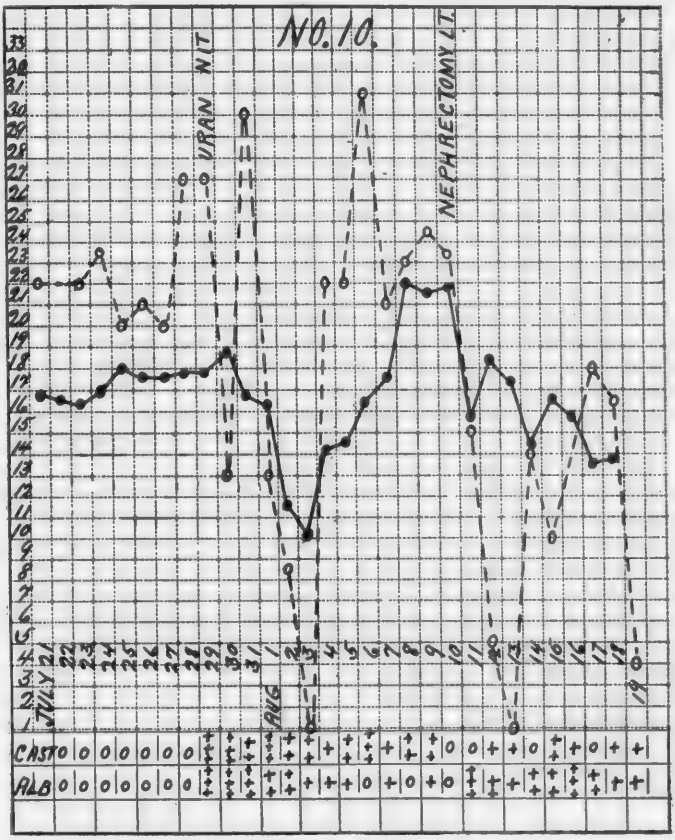

FIG. 13.

Rabbit No. 10.

	Weight.	Seconds.							
		15	30	45	60	75	90	105	120
July 21	1980 grams	16.8	21.2	25.4	29.2	32.8	36.4	38.8	40.6
" 22	1980 "	16.2	20.4	23.8	26.2	37.8	36.6	39	41
" 23	1870 "	17	22.4	26.6	30.6	33.6	36.2	39.6	42.2
" 24	1880 "	18	23.2	26.8	30.2	33.4	36.6	39.6	42
" 25	1930 "								
" 26	1940 "	17.6	22.6	26.4	30	33.6	35	40	42.4
" 27	2030 "	17.6	21.8	25.6	29.2	32.6	35.6	38.4	40.6
" 28	1900 "								
" 29		17.8	23.6	27.6	31	35	38.6	42.4	45.6
	5 m. 1 per cent uranium nitrate solution.								
" 30	1920 grams	18.8	23	26.8	31.6	35	38.4	41.2	43
" 31	1900 "	16.4	20.2	23.6	27	30.4	33.4	36.6	38.8
August 2	1050 "	11.6	14.6	17.6	19.8	22.4	24.6	27	29.6
" 3	1970 "	10.4	13.8	16.2	19	21.2	23.4	26	27.8
" 4	1720 "	14.4	18.6	22.6	26	29.6	32.8	35.4	37.8
" 5	1660 "	14.4	18.6	22	25.4	28.6	32	34.8	36.6
" 6	1490 "	16.6	21.2	25.6	29.4	32.4	35.6	35.6	41.2
" 7	1520 "	17.6	23	27.8	32	35.6	38.6	41.2	
" 8	1570 "	22	28.4	33	37.4	41	44.4	47.2	49.6
" 10	1700 "	21.8	27.2	31.8	36	39.6	43	45.6	48
Operation—Left nephrectomy									
August 11	1700 "	15.8	20	23	25.6	28.8	31.4	34	36.2
" 12	1570 "	18.2	24.4	29	33.4	37.4	41	44	46
" 13	1520 "	17.2	22.4	26	31	34.8	38.4	41	43.6
" 14	1350 "	14.4	18.6	21.8	25	28.6	31	33.4	35.4
" 15	1320 "	16.4	22.6	26.4	30.2	34	37.6	40	42.8
" 16	1340 "	15.8	20.6	24.6	28.4	31.6	34.8	37.4	39.6
" 17	1350 "	15.6	17.4	20.2	22.8	25.6	28.6	31	33.2

There are several points of interest connected with this case. *First.*—With the suppression of urine and the onset of uræmia there was a decided drop in the catalytic activity of the blood. The same curve was obtained here as in cases with suppression of urine consequent upon ligation of the ureters. As soon as the kidneys again began to function the catalytic activity of the blood rose. It is of importance to note, however, that this curve follows the condition of the animals much more closely than the urinary findings. The urinary output varied markedly while the albumin and casts were in abundance, but these latter diminished rather than increased before the onset of uræmia. In Rabbit No. 13 this picture was exactly reproduced. *Second.*—Following the attack of uræmia the catalytic activity of the blood was greater than it had been before an injection of uranium nitrate. This has been repeatedly observed and has been referred to under Rabbit No. 18. Its sig-

Urinary Chart.

	Amount.	Sp. Gr.	Reaction.	Albumin.	Microscopic Examination.
July 23	220 c.c.	1010	Acid	0	No pathological elements.
" 24	235 "	1012	Alk.	"	" " "
" 25	200 "	1010	"	"	" " "
" 26	210 "	1010	"	"	" " "
" 27	200 "	1012	"	"	" " "
" 28	270 "	1014	"	"	" " "
" 29	170 "	1014	"	"	" " "
" 30	130 "	1010	"	++	Casts: hyaline, waxy, granular. Renal epithelial cells.
" 31	300 "	1012	"	"	No pathological elements.
Aug. 1	130 "	1020	"	"	" " "
" 2	85 "	1004	"	"	" " "
" 3	0 "				
" 4	220 "	1010	"	+++	" " "
" 5	220 "	1012	"	+	" " "
" 6	310 "	1005	"	"	" " "
" 7	210 "	1015	"	"	" " "
" 8	230 "	1016	"	"	" " "
" 10	235 "	1014	"	0	No casts; few epithelial cells
" 11	150 "	1010	"	"	" " " " " "
" 12	50 "	1012	"	++	" " " " " "
" 13	0 "				
" 14	145 "	1002	"	+	" " " " " "
" 15	100 "	1010	"	+	" " " " " "
" 16	190 "	1012	"	++	Hyaline casts.
" 17	175 "	1018	"	+	" "
" 18	40 "	1010	"	"	" "

nificance is not known. *Third.*—The condition of the kidney showed that there was sufficient renal degeneration to account for the symptoms, and its low catalytic activity was further proof of the animal's poor general condition. *Fourth.*—The removal of one kidney threw an excess of work on the remaining diseased organ which this was unable to perform. This insufficiency manifested itself by the immediate fall of the catalytic activity of the blood. The output of urine likewise fell, and showed an increased content of albumin and casts. But while the catalytic activity tended to fall in a continuous curve until death, the output of urine varied from day to day and showed no constant increase in the amount of its pathological contents.

Group 3.—In this group the animals are included which survived repeated injections of uranium nitrate but finally died of chronic nephritis. Rabbit No. 7 will be given as an illustration. The following notes are abstracted from the history.

Rabbit No. 7.—On July 16, the animal received the first dose of uranium nitrate, 10 m of a one per cent. solution being injected subcutaneously. After

thirty-six hours it became languid and showed a decrease in its urinary excretion, which contained a large amount of albumin and casts. The catalytic activity of its blood which had been quite constant became irregular. This condition continued until July 21, when the urinary excretion again increased in amount, and, although varying somewhat, assumed a slightly higher level than it had

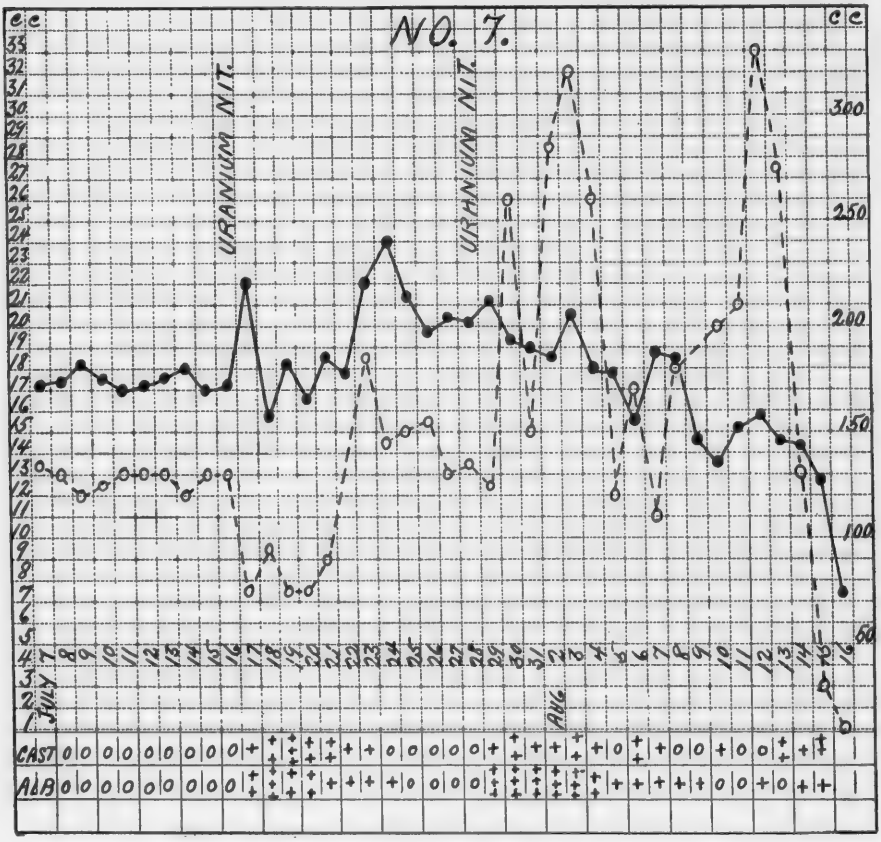

FIG. 14.

had previous to the injection. The albumin and casts decreased in amount and were entirely absent on July 24. The catalytic activity of its blood became higher and remained so, though slightly irregular. The condition of the animal improved considerably so that on July 28 it was thought permissible to give the second injection of 10 m of uranium nitrate. Following the second injection the catalytic activity of the animal's blood gradually declined

until its death. The output of urine, however, became very irregular; on the whole there was a much greater daily output than previously, and although at first the urine contained a large number of casts and considerable albumin, these were not constant findings and tended to decrease in amount.

The animal's general condition gradually declined and although it ate considerably and seemed lively there was a steady loss of weight. It died on August 16, thirty-three days after the first injection of uranium nitrate. At autopsy the body was markedly emaciated; the kidneys were very small and of a pale yellowish color. The capsule striped readily leaving a smooth mottled surface, yellowish opaque lines criss-crossing between the pale brown areas. On section the cortex was seen to be markedly shrunken and varied considerably in thickness. The striations were distinct but not quite regular. Here and there a faint grayish streak of fibrous tissue was seen in the cortex and yellowish opaque lines could be made out throughout the parenchyma. The glomeruli were distinct as pink pin point dots. The pyramids were very pale.

Microscopically the cortex was extremely thin and the tubules shrunken together; their epithelium was vacuolated and desquamated in places and the lumina of the tubules showed large clear-staining droplets and few casts. The Malpighian corpuscles were small and showed a distinct inter-capillary glomerulitis. Everywhere one could see a small increase of fibrous tissue between the tubules and in places this was slightly more extensive, compressing the tubules in its vicinity.

The catalytic activity of the kidney and liver was as follows:

	Seconds.							
	15	30	45	60	75	90	105	120
Kidney	10.4	15.8	19.9	23.4	27.2	31	34.2	37
Liver	17.2	23.4	28.4	32.6	36.4	40.4	43.6	46

The above experiment is significant for the following reasons: *First.*—The urinary excretion and the catalytic activity of the blood were both practically constant before the uranium nitrate injection. Following this, however, there was a temporary fall in both. *Second.*—After the appearance of the nephritis neither the output of urine nor the catalytic activity of the blood was constant; both showed marked variations from day to day. It is interesting to note in this connection that the only rabbit whose blood did not show a constant catalytic activity showed at autopsy spontaneous nephritis. *Third.*—After the second injection of uranium nitrate there was again a fall from the high position the catalase had taken, and this fall continued with slight variations from day to day. This is the more important since the urine did not show any

constant change; the amount was relatively greater than before the first injection and the presence of albumin and casts was inconstant. Therefore, though the kidneys may have been acting as competent filters something seemed to be retained in the blood which normally is removed by the kidney, and this retention manifested itself by the decrease of the catalytic activity of the blood. The conclusion seems justified that the functional sufficiency of the kidney manifests itself under certain conditions better in the catalytic activity of the blood than in the urinary findings.

The application of this work to clinical medicine is now in progress in the Johns Hopkins Hospital.

Urinary Chart.

	Amount.	Sp. Gr.	Reaction.	Albumin.	Microscopic Examination.
July 7	135 c.c.	1008	Alk.	0	No pathological elements.
" 8	130 "	1010	"	"	" " "
" 9	130 "	1008	"	"	" " "
" 12	125 "	1010	"	"	" " "
" 14	130 "	1008	"	"	" " "
" 15	130 "	1009	"	"	" " "
" 16	120 "	1010	"	++	Casts: granular and hyaline. White blood cells.
" 17	75 "	1006	"	++	Ditto.
" 18	95 "	1010	"	"	Ditto.
" 19	75 "	1010	"	"	Ditto.
" 20	75 "	1010	"	"	Ditto.
" 21	90 "	1012	"	+	Few casts.
" 23	185 "	1006	"	"	" "
" 24	145 "	1008	"	0	Negative.
" 25	90+ "	1010	"	"	"
" 26	75 "	1014	"	"	"
" 27	140 "	1014	"	"	"
" 28	135 "	1012	"	"	"
" 29	140 "	1010	"	"	"
" 30	125 "	1012	"	+++	Casts; hyaline and granular. Renal epithelium.
" 31	260 "	1010	"	"	Ditto.
Aug. 1	150 "	1014	"	"	Ditto.
" 2	285 "	1010	"	"	Ditto.
" 3	320 "	1008	"	++	Few casts.
" 4	260 "	1012	"	"	" "
" 5	120 "	1015	"	+	" "
" 6	170 "	1007	"	"	Negative.
" 7	110 "	1017	"	"	"
" 8	185 "	1010	"	"	Numerous hyaline and granular casts.
" 10	210 "	1006	"	0	Few casts.
" 11	200 "	1010	"	"	" "
" 12	360 "	1007	"	"	" "
" 13	275 "	1004	"	+	Negative.
" 14	130 "	1012	"	"	"
" 15	30 "	1004	"	"	Numerous casts.
" 16	0 "		"		

	Weight.	Seconds.							
		15	30	45	60	75	90	105	[illegible]
July 1	1830 grams	17.4	24	24.2	33	36.6	39.8	42	44.4
" 2	1750 "	17	23.6	27.8	32	35.8	39	42	44.4
" 3	1800 "								
" 4	1770 "	17	23	28	32.4	36.2	39.4	42.6	45
" 5	1690 "	16.2	21.2	25.4	29	32.6	35.4	37.8	40
" 6	1680 "	17.2	24.2	29	32.4	36.4	40	42.4	45.2
" 7	1730 "								
" 8	1750 "	17.4	23.8	28.6	33	37	39.6	41.8	44.8
" 9	1760 "	18.2	24.6	29.6	34	37.4	41	43.6	46.2
" 10	1780 "	17.6	23.8	28.6	33	37.8	40.2	43	45.4
" 11	1730 "	17	22.6	27.6	32.8	36.8	39.2	40	43.2
" 12	1750 "	17.2	23.4	26.8	32.6	36.6	39.8	41.6	44.8
" 13	1780 "	17.4	24.2	28.2	33.6	38	40.2	42.6	45.4
" 14	1750 "	18	24.4	27.8	33	37.4	39.2	43	45
" 15	1725 "	17	22.8	26	32.4	36.2	38.4	40.8	42.8
" 16	1740 "	17.2	23	28	34	38.2	40.8	42	45
" 16	10 ♏ 1 per cent. uranium nitrate solution								
" 17	1600 grams	22	28	33	37.2	41	44.4	47	49.4
" 18	1520 "	15.8	20.4	24.6	28	31.8	35	38	40.2
" 19	1410 "	18.4	25.6	29	33	37.6	40.2	42.2	45.4
" 20	1530 "	16.6	21.6	25.6	29.8	33.2	37	39.4	41
" 21	1540 "	18.6	23.8	28	33.2	36.8	40.2	43	48.6
" 22	1500 "	17.6	21.4	24.2	27	30.4	33.2	35.8	38.2
" 23	1470 "	22	28	33.8	38.6	43.4	46.4	48.8	50.4
" 24	1500 "	24	30.8	36.2	41.4	45.6	48.8	50.6	52.
" 25	1600 "	21.2	27.8	32.8	37.4	41.2	44.6	47.2	49.4
" 26	1710 "	19.8	25.2	31.6	35.4	37	40.2	43	46
" 27	1700 "	20.4	27	32.4	36.6	39	42.4	45.6	48.2
" 28	10 ♏ 1 per cent. uranium nitrate solution								
" 29	1560 grams	21.2	28.8	33	36.6	39	41	45.4	46
" 30	1470 "	19.4	25.4	30.6	34.4	38.2	41.4	44.2	46.3
" 31	1350 "	19	24.4	27.8	32.4	37.8	40.4	43.2	46
August 2	1350 "	18.6	24	28.4	32.2	36.4	40	43.8	46
" 3	1330 "	20.6	26.4	31.2	35.4	39.6	43	46	48.6
" 4	1200 "	18	22.2	26.6	31.2	34.8	38.6	41.6	44.2
" 5	1190 "	17.8	22.6	26.6	30.8	34.6	38.2	40.8	43
" 6	1170 "	15.6	20	24	27.2	30	32.8	35.4	37.6
" 7	1165 "	18.8	25.4	30.2	37.2	30	32.8	35.4	37.6
" 8	1140 "	18.8	23.8	27	34.2	38	41.4	44	46.6
" 9	1200 "	14.6	18.8	22.8	26.6	29.2	31.8	33.8	36
" 10	1160 "	13.6	16.8	18.8	21	24	26	27.8	29
" 11	1140 "	15.4	19.2	22.4	25.4	28.6	31.4	34.2	36.6
" 12	1150 "	15.8	19.8	23	27.6	30	33.6	35.4	39.2
" 13	1150 "	14.6	19	23	26.4	29.8	32.6	34.6	37
" 14	1080 "	14.6	19.2	23.6	27	30.2	33	35.4	38
" 15	1000 "	12.8	16.6	19.8	22.6	25.8	28.4	31	32.2
" 16	1020 "	7.4	9.4	10.6	12.4	15	16.6	17.8	19

CONCLUSION.

1. The inhibiting action of urine on catalase depends to a great extent on its reaction. The urine of a nephritic has no greater inhibiting power than normal urine provided the reaction of urines tested is neutral.

2. The catalytic activity of a single rabbit's blood is constant from day to day.

3. There is considerable variation in the catalytic action of the blood of different rabbits.

4. Following ligation of the ureters the catalytic activity of the blood gradually decreases; the tissues of an animal which has died as a result of ligation of the ureters show a decided decrease in catalytic activity, when compared with those of normal animals.

5. Following bilateral nephrectomy the same decline of the catalytic action of the blood is observed as after bilateral ligation of ureters.

6. Following unilateral nephrectomy the catalytic activity of the blood may temporarily fall and then rise above its previous level, or it may fall continuously. Wherever the continuous fall occurs the animal dies.

7. When the kidneys are allowed to function but the urine is drained into the peritoneal cavity there is no change in the catalytic activity of the tissues post-mortem but a marked rise in the catalytic activity of the blood accompanies the resulting peritonitis.

8. The same rise in the catalytic activity of the blood is obtained in experimental bacterial peritonitis. This might be of diagnostic importance in determining early inflammations of the peritoneum.

9. A kidney functioning normally changes substances circulating in the blood into urinary products devoid of any influence on catalase. If the kidney cannot effect this change, these substances remain unaltered in the circulating blood and by their presence inhibit the catalytic activity of the blood and organs.

10. *Nephritis* (Uranium Nitrate).—*A.* In acute nephritis there is a marked decrease in the catalytic activity of the blood and of the tissues post-mortem. The decrease in the catalytic activity of the blood may vary directly with the amount of urine excreted.

B. With the onset of uræmia the catalytic activity of the blood decreases markedly and follows in a general way the urinary findings, especially the total amount of urine. If the animal recovers the catalase gradually rises.

C. Although the catalytic activity of a normal rabbit's blood is constant from day to day, the activity of blood from a rabbit in

which an experimental nephritis has been produced oscillates markedly. The catalytic activity of the blood may under certain conditions indicate the functional sufficiency of the kidneys much more accurately than the urinary findings.

Note.—Throughout the paper I have frequently mentioned "decrease catalytic activity of the blood and organs." I do not wish to presuppose with this statement the diminution of the amount of catalase in the blood and tissues, but only wish to point out that in the experiments the conditions were altered in such a way that the catalase displayed in some instances increased and in others diminished activity. From the experiments of Amberg and Loevenhart[4] it becomes evident that a greater or lesser activity of a ferment mixture does not permit a conclusion as to the actual amount of ferment present, since inhibiting or accelerating substances may exercise a determining influence on the ferment activity.

[4] Amberg and Loevenhart, *Jour. of Biol. Chem.* 1908, iv, 149.